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Acetylation of Histones and Transcription-Related Factors

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INTRODUCTION

Eukaryotic transcription is a highly regulated process, and acetylation is now known to play a major role in this regulation. Specifically, acetyltransferase enzymes that act on particular lysine side chains of histones and other proteins are intimately involved in transcriptional activation. By modifying chromatin proteins and transcription-related factors, these acetylases are believed to regulate the transcription of many genes.

Chromatin structure, the way in which DNA is packaged in the eukaryotic cell, is known to have a major impact on levels of transcription. In eukaryotes, DNA typically exists in vivo as a repeating array of nucleosomes (271), in which 146 bp of DNA are wound around a histone octamer (consisting of two each of histone proteins H2A, H2B, H3, and H4). Nucleosomes are the first level of chromatin organization, although they in turn are organized into higher-order structures of increasing complexity (129), an extreme example being the condensed metaphase chromosome during cell division. A number of studies have demonstrated that nucleosomal DNA is generally repressive to transcription (91, 183); thus, nucleosome structure and DNA-histone interactions typically make the DNA of genes and their regulatory regions unavailable for the binding of the transcriptional machinery and other factors involved in activation. The direct connection between chromatin alteration and transcriptional activation has been increasingly demonstrated in recent years.

Certain enzymes and protein complexes are now known to bring about changes in the state of chromatin by numerous mechanisms, with resultant effects on gene expression. One class of complexes alter the DNA packaging (remodel chromatin) in an ATP-dependent manner; these include the Swi-Snf complex and a number of others from various organisms (114, 126). Another class of chromatin-altering factors act by covalently modifying histone proteins. These modifications can include phosphorylation, ubiquitination, ADP-ribosylation, and methylation (25), but the best-characterized mechanism is acetylation, catalyzed by histone acetyltransferase (HAT) enzymes.

HATs function enzymatically by transferring an acetyl group from acetyl-coenzyme A (acetyl-CoA) to the ε-amino group of certain lysine side chains within a histone's basic N-terminal tail region (149). Within a histone octamer, these regions extend out from the associated globular domains, and in the context of a nucleosome, they are believed to bind the DNA through charge interactions (positively charged histone tails associated with negatively charged DNA) or mediate interactions between nucleosomes (67, 151). Lysine acetylation, which neutralizes part of a tail region's positive charge, is postulated to weaken histone-DNA (107, 221) or nucleosome-nucleosome interactions (68, 152) and/or signal a conformational change (175), thereby destabilizing nucleosome structure or arrangement and giving other nuclear factors, such as the transcription complex, more access to a genetic locus. In agreement with this is the fact that acetylated chromatin has long been associated with states of transcriptional activation (99, 244). Recently, some of the proteins and complexes that carry out these acetylation functions have been characterized, and they will be discussed in this review. Interestingly, certain HATs have also recently been shown to specifically acetylate lysine residues within transcription-related proteins other than histones; these events and their regulatory potential will be discussed as well.

Finally, histone acetylation is a reversible process, and deacetylases are also integral to cycles of transcription. Acetylation is generally associated with activation, whereas lack of acetylation tends to correlate with repression—two regulatory processes working in harmony to achieve appropriate levels of transcription (135). While outside the scope of this review, it should be noted that a number of deacetylase proteins and complexes have been characterized in the last several years. This has provided a further conceptual linkage between acetylation and transcriptional activity, since some of the histone deacetylases (HDACs) and the proteins with which they associate are previously known DNA-binding repressors or corepressors (reviewed in reference 186).

HISTONE ACETYLTRANSFERASES (HATs)

The phenomenon of histone acetylation in the eukaryotic cell has been known for many years, and since the early 1970s various HAT activities have been isolated and partially characterized. Each of these enzymes generally belongs to one of two categories (30, 74): type A, located in the nucleus, or type B, located in the cytoplasm, although recent evidence indicates that some HAT proteins may function in multiple complexes or locations and thus not precisely fit these historical classifications (200). B-type HATs are believed to have somewhat of a housekeeping role in the cell, acetylating newly synthesized free histones in the cytoplasm for transport into the nucleus, where they may be deacetylated and incorporated into chromatin (4, 199). The A-type HATs, on the other hand, acetylate nucleosomal histones within chromatin in the nucleus; these HATs are potentially linked to transcription and thus are the main focus of this review. A summary of known HAT proteins is presented in Table 1, and these are discussed further in the text.

GNAT Superfamily

The best-understood set of acetyltransferases is the GNAT (Gcn5-related N-acetyltransferase) superfamily (174), which have been grouped together on the basis of their similarity in several homology regions and acetylation-related motifs (Fig. 1A). This group includes the HAT Gcn5, its close relatives, and at least three more distantly related HATs, Hat1, Elp3, and Hpa2. It also contains a variety of other eukaryotic and prokaryotic acetyltransferases with different substrates, indicating the conservation and wide application of this type of acetylation mechanism throughout evolution. Four sequence motifs whose functions are not yet fully understood—C, D, A, and B, in N-terminal to C-terminal order—define this superfamily. The C motif is found in most of the GNAT family acetyltransferases but not in the majority of known HATs. Motif A is the most highly conserved region, and it is shared with another HAT family, the MYST proteins, described later in this review. Furthermore, it contains an Arg/Gln-X-X-Gly-X-Gly/Ala segment that has been specifically implicated in acetyl-CoA substrate recognition and binding (59, 270).

Gcn5. The first protein identified as an A-type, transcription-related HAT was discovered in the ciliate *Tetrahymena thermophila* (31). By way of an in-gel assay of nuclear extract chromatographic fractions run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a 55-kDa polypeptide (p55) was found to have acetylation activity on free histones (29). Subsequent protein sequencing revealed that it was a homolog of *Saccharomyces cerevisiae* (yeast) Gcn5 (77), previously identified as a transcriptional adaptor (or coactivator) involved in the interaction between certain activators and the transcription complex (17, 154, 213). Homologs of Gcn5 have more recently been cloned and sequenced from numerous divergent organisms—such as human (36), mouse (276),

TABLE 1. Summary of known and putative HATs

HAT	Organisms known to contain the HAT	Known transcription-related functions/effects	HAT activity demonstrated in vitro[b]	Histone specificity of recombinant enzyme in vitro[a,b]	Known native HAT complexes and nucleosomal histone specificities in vitro
GNAT superfamily					
Hat1	Various (yeast to humans)	None (histone deposition-related B-type HAT)	Yes	**H4**	Yeast HAT-B, HAT-A3 (no nucleosome acetylation)
Gcn5	Various (yeast to humans)	Coactivator (adaptor)	Yes	**H3**/H4	Yeast ADA, SAGA (**H3**/H2B); human GCN5 complex, STAGA, TFTC (**H3**)
PCAF	Humans, mice	Coactivator	Yes	**H3**/H4	Human PCAF complex (**H3**/weak H4)
Elp3	Yeast	Transcript elongation	Yes	ND*	Elongator, polymerase II holoenzyme (**H3**/weak H4)
Hpa2	Yeast	Unknown	Yes	**H3**/H4	
MYST family					
Sas2	Yeast	Silencing	ND		
Sas3	Yeast	Silencing	Yes	**H3**/**H4**/H2A	NuA3[c] (**H3**)
Esa1	Yeast	Cell cycle progression	Yes	**H4**/H3/H2A	NuA4 (**H4**/H2A)
MOF	*Drosophila*	Dosage compensation	Yes	**H4**/H3/H2A	MSL complex (**H4**)
Tip60	Humans	HIV Tat interaction	Yes	**H4**/H3/H2A	Tip60 complex
MOZ	Humans	Leukemogenesis, upon chromosomal translocation	ND		
MORF	Humans	Unknown (strong homology to MOZ)	Yes	**H4**/H3/H2A	
HBO1	Humans	ORC interaction	Yes*	ND*	HBO1 complex
p300/CBP	Various multicellular	Global coactivator	Yes	**H2A/H2B/H3/H4**	
Nuclear receptor coactivators		Nuclear receptor coactivators (transcriptional response to hormone signals)			
SRC-1	Humans, mice		Yes	**H3**/H4	
ACTR	Humans, mice		Yes	**H3**/**H4**	
TIF2	Humans, mice		ND		
$TAF_{II}250$	Various (yeast to humans)	TBP-associated factor	Yes	**H3**/H4	TFIID
TFIIIC		RNA polymerase III transcription initiation			TFIIIC (**H2A**/**H3**/**H4**)
TFIIIC220	Humans		Yes*	ND	
TFIIIC110	Humans		Yes	ND	
TFIIIC90	Humans		Yes	**H3**	

[a] Histones that are the primary in vitro substrates for a given HAT are bold; other histones listed are acetylated weakly or in a secondary manner.
[b] Asterisks indicate proteins for which HAT activity has been suggested indirectly or demonstrated in an incomplete manner. Elp3 can acetylate all four histones but has only been tested with them individually in in-gel assays. The HAT function of HBO1 has primarily been shown by the in vitro free histone **H3**/H4-acetylating activity of a purified human complex containing it, although recombinant GST-HBO1 (and the complex) did weakly acetylate nucleosomes. Finally, TFIIIC220 was identified as a HAT only in in-gel assays, and its activity has not been confirmed by recombinant protein studies as of this writing. ND, not determined.
[c] S. John and J. L. Workman, unpublished result.

Schizosaccharomyces pombe, *Drosophila melanogaster* (215), *Arabidopsis thalania*, and *Toxoplasma gondii* (102)—suggesting that its function is highly conserved throughout the eukaryotes.

To date, yeast Gcn5 (general control nonderepressible-5; also referred to as yGcn5) is the best characterized of the HATs, both structurally and functionally and both in vivo and in vitro. Various studies have mapped and characterized the functional domains of yeast Gcn5, shown in Fig. 1B (35, 37). These include a C-terminal bromodomain, an Ada2 interaction domain, and the HAT domain, which by use of truncation mutants was found to be required for adaptor-mediated transcriptional activation in vivo (37). The Gcn5 HAT domain was also functionally analyzed by alanine scan mutagenesis. These analyses identified conserved residues critical to HAT activity and demonstrated the direct correlation of Gcn5 HAT function with cell growth, in vivo transcription, and histone acetylation at the Gcn5-dependent *HIS3* promoter in vivo (134, 256). A further study with some of these mutants showed that Gcn5's HAT activity has an effect on chromatin remodeling at the *PHO5* promoter in vivo (89).

The substrate specificity of Gcn5 has also been investigated. In vitro, recombinant Gcn5 was found to acetylate histone H3 strongly and H4 weakly in a free histone mixture (although histone H4 was acetylated well individually). Protein sequence analysis of these reaction products revealed that the primary sites of acetylation were lysine 14 on histone H3, as shown in Fig. 2, and lysines 8 and 16 on histone H4 (136). Although recombinant Gcn5 can acetylate free histones efficiently, it is unable to acetylate nucleosomal histones (84, 136, 201), the more physiological substrate, except under special conditions and at high enzyme concentrations (243). Only in the context of multisubunit native complexes such as SAGA and ADA (described later in this review) is Gcn5 able to acetylate nucleosomes effectively, indicating that the influence of other proteins is required to confer this activity.

In mammals (humans and mice), the Gcn5 subclass of acetyltransferases is represented by two closely related proteins, GCN5 and p300/CREB-binding protein-associated factor (PCAF). These proteins share a remarkable degree of homology (about 70% identity and 80% similarity) throughout their sequences, and a distinguishing feature is an approximately 400-residue amino-terminal region not present in yeast Gcn5 (Fig. 1B) (276); such an extension is seen, however, in *Drosophila* GCN5 (215). The function of human GCN5 (also known as hGCN5) has also been investigated in vitro and in vivo, and it was found to carry out transcriptional adaptor roles analogous to those of yeast Gcn5 (36). Further studies showed that human GCN5 had HAT activity in vitro (279) and that its

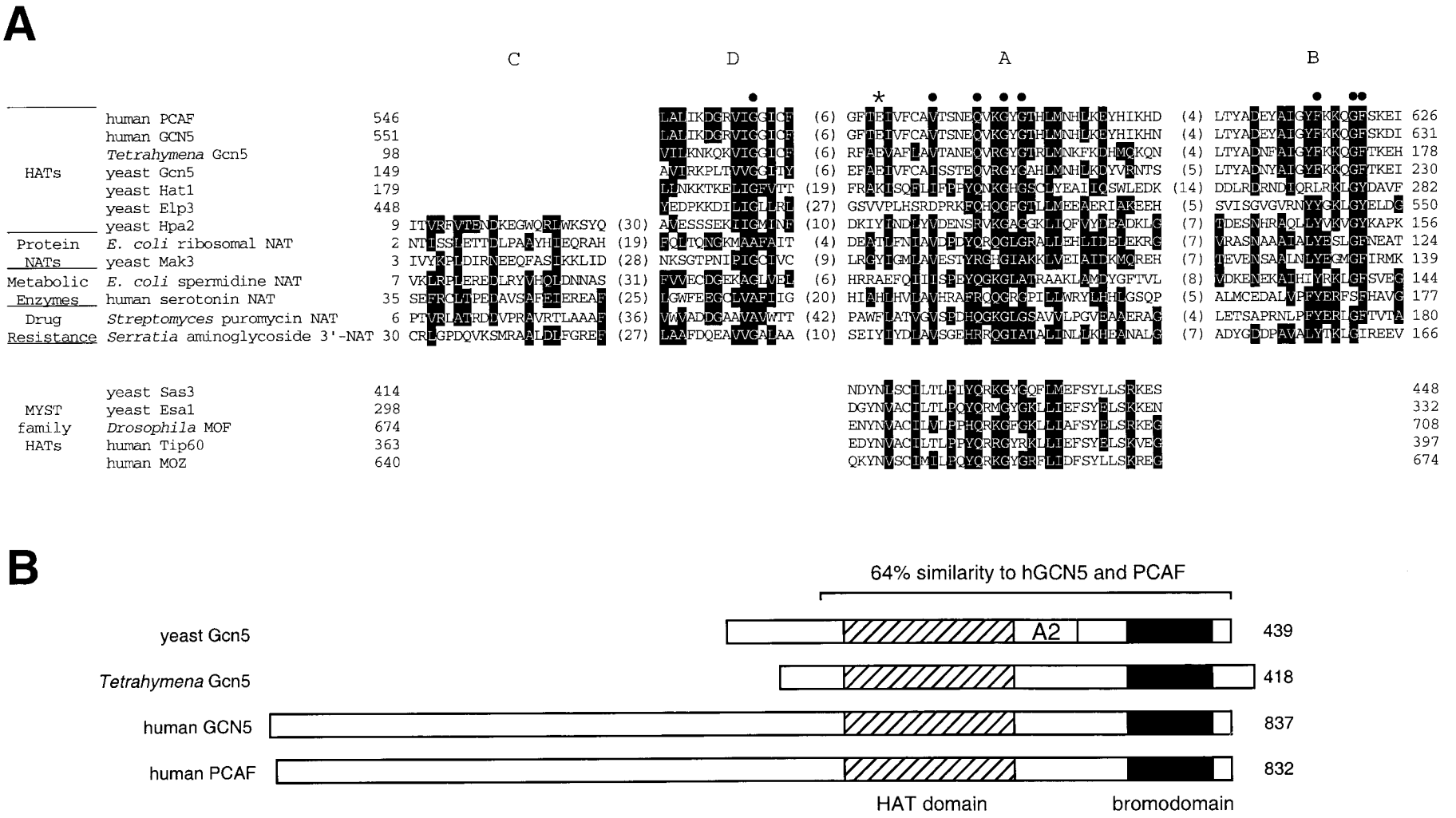

FIG. 1. Similarities of GNAT (Gcn5-related *N*-acetyltransferase) superfamily members. (A) Alignment of GNAT homology motifs A, B, C, and D for HATs and representatives of other types of acetyltransferases. Reversed type indicates consensus sequence residues, as determined by Neuwald and Landsman (174), and solid circles mark residues that are particularly conserved throughout the superfamily. The asterisk indicates the glutamate residue known to be critical for HAT catalysis in yeast Gcn5. At the bottom are several members of another acetyltransferase family, the MYST proteins, that share just the A motif. (B) Alignment of proteins from the Gcn5 subgroup of the GNAT superfamily, showing the location of the HAT domain and bromodomain. Shown at the top is the overall homology region shared by all four proteins, with the similarity between the yeast and human proteins indicated; *Tetrahymena* Gcn5 has 62% similarity with the three others over this same region. The A2 label designates a region in yeast Gcn5 known to interact with the adaptor protein Ada2 (37). In addition, the N-terminal region of PCAF has been generally defined as its site of interaction with p300/CBP and nuclear receptor coactivator SRC-1 (130, 276); this region and the HAT domain also interact with viral protein E1A (40, 193).

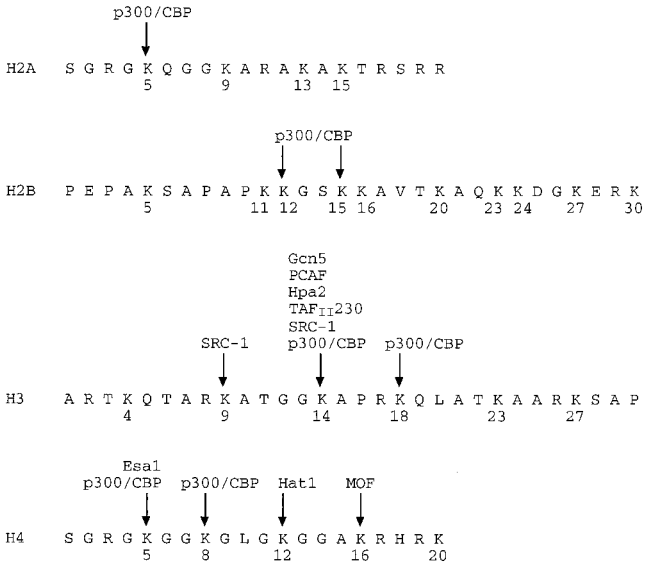

FIG. 2. Primary histone acetylation specifities of some of the known HAT proteins in vitro. Shown are the amino acid sequences for the N-terminal tail regions of human histones H2A, H2B, H3, and H4, with lysine residues numbered and arrows indicating the predominant sites used by various HATs in in vitro experiments. $TAF_{II}230$ is the *Drosophila* homolog of human $TAF_{II}250$ used in site specificity determinations. The above H3 and H4 sequences are nearly identical to those of *S. cerevisiae* and *Drosophila*. Specific but relatively nonpreferred or minor sites for certain HATs are not indicated, for example, H4 lysine-8 for Gcn5 and PCAF. It should be noted that lysine specificities may be somewhat expanded or restricted with native HAT complexes and/or on nucleosomal substrates.

HAT domain could successfully substitute for that of yeast Gcn5 in vivo, indicating the evolutionary conservation of this HAT function (257).

The HAT domain of human GCN5 is of course indispensable to its acetylation function, but interestingly, two other domains appear to have an influence on its HAT activity and substrate use. Because of the apparent existence of multiple alternatively spliced versions of human *GCN5*, the original cDNA clones lacked its N-terminal region. While recombinant short-form human GCN5 could acetylate histone H3 (and to a lesser extent H4) only as free histones (257, 279), the full-length forms of human and mouse GCN5 were recently shown to be competent for the acetylation of nucleosomal histones, implicating the N-terminal region in chromatin substrate recognition (276). The C-terminal bromodomain is another region that apparently has an effect on human GCN5 HAT function, interacting with the DNA-dependent protein kinase holoenzyme, which inhibits GCN5's HAT activity by way of phosphorylation (11). Additional functional aspects of the bromodomain are discussed below.

PCAF. The gene for PCAF (also referred to as P/CAF) was originally identified from a human cDNA database on the basis of its homology to Gcn5. Because of functional similarities between the yeast activator Gcn4 (which interacts with the adaptor complex) and the activator c-Jun in higher eukaryotes (which interacts with coactivators p300 and CREB-binding protein [CBP]), it was postulated that a human counterpart of Gcn5 may participate in p300/CBP-mediated activation. When PCAF was cloned and investigated, in vitro and in vivo studies revealed that it interacts with p300 and CBP (279), hence its name. p300 and CBP are very closely related coactivators that mediate the transcription of many genes and are also HATs, as described below. PCAF HAT activity, like full-length GCN5, in recombinant form acetylates either free histones or nucleosomes (279), primarily on lysine-14 of histone H3, and more weakly on lysine-8 of histone H4 (207).

Relevant to PCAF function is the fact that it binds to the same site on p300/CBP as does adenoviral oncoprotein E1A, and competition between these two proteins was observed (279). Interestingly, transfected PCAF and E1A had opposite effects on cell cycle regulation, suggesting that PCAF has a role in inhibiting cell cycle progression and that E1A's mitogenic activity may occur by disrupting the interaction between PCAF and p300/CBP (279). In addition, E1A and the regulatory protein Twist reduce PCAF-mediated in vivo transcription by binding to PCAF, further identifying this acetyltransferase as a target for regulation. Twist may function by inhibiting PCAF's HAT activity (96); a similar HAT-inhibitory effect was observed for E1A in two studies (40, 96) but not another (193), so it will be important to clarify the generality of HAT inhibition.

The role of PCAF in transcription has been investigated by multiple studies, and its requirement as a HAT and coactivator has been described for myogenesis (192) and nuclear receptor-mediated (21, 130) and growth factor-signaled (275) activation, among other processes. Furthermore, a reporter gene study demonstrated that PCAF could carry out its coactivator function in a HAT-dependent manner and stimulate transcription when bound either to a promoter-proximal site or at a distant enhancer (132). Although PCAF was originally characterized as a HAT, much recent work has focused on its acetylation of various nonhistone transcription-related proteins. These include the chromatin proteins HMG17 and HMG I(Y), activators p53, MyoD, and human immunodeficiency virus (HIV) Tat, and general transcription factors TFIIE and TFIIF. These activities and their potential regulatory significance are described later in this review. At present, it appears likely that both types of activities, HAT and factor acetyltransferase (FAT), are physiologically important for PCAF function.

Finally, there are several noteworthy similarities and differences between PCAF and GCN5. One similarity is that in human cells, each participates in separate SAGA-related multisubunit complexes (described below) whose subunits are otherwise largely identical (177). Also, like PCAF, human and mouse GCN5 bind p300/CBP, suggesting functional similarity, although the precise sites bound may be different for each binding pair (276). A further difference between PCAF and GCN5 is that while both are ubiquitously expressed in the mouse, their comparative levels were very different in many tissues (276). Future studies will be required to determine if PCAF and GCN5 are functionally redundant or distinct.

Hat1, Elp3, Hpa2, and other acetyltransferases. Gcn5, its homologs, and PCAF have high sequence similarity, but as members of the GNAT superfamily, they are also related by sequence motifs to other HATs and numerous nonhistone acetyltransferases, even prokaryotic ones (174). As shown by the abbreviated list in Fig. 1A, these include the yeast HATs Hat1, Elp3, and Hpa2, protein *N*-acetyltransferases (which modify N-termini), metabolic enzymes, acetylases involved in drug resistance and detoxification, and a variety of other proteins with unknown specific functions. In addition, GNAT homology is seen in several known transcriptional regulators for which acetylase activity has not yet been described—the yeast Spt10 protein (173), for example, which affects the expression of various genes (172, 278), including certain histone genes (55).

The first HAT protein to be identified was actually yeast Hat1 (127, 185), originally described as a B-type HAT involved in the cytoplasmic acetylation of histones destined for deposition on DNA in the nucleus. Hat1 is responsible for the predominant cytoplasmic HAT activity in *S. cerevisiae*, although a

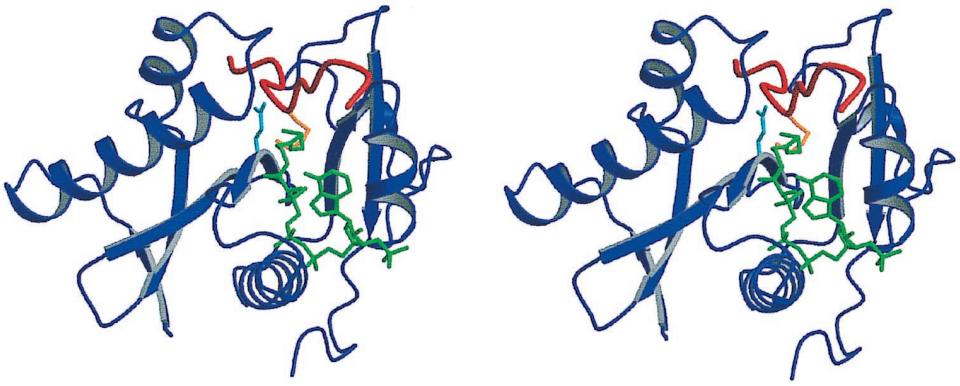

FIG. 3. Stereo diagram of the structure of a HAT domain bound to its substrates. Shown is the GNAT superfamily protein *Tetrahymena* Gcn5 (blue) with a histone H3 N-terminal tail peptide (red) and CoA (green) bound to its upper and lower clefts, respectively. At the active site, the glutamate-122 residue (aqua), analogous to yeast Gcn5 glutamate-173, catalyzes the transfer of an acetyl group from acetyl-CoA to the lysine-14 sidechain (orange) of H3 peptide. The N termini of both the Gcn5 protein and the H3 peptide are to the left in diagram, and C-termini are to the right.

null mutation of its gene confers no phenotype, suggesting that its function may be redundant with other HATs. Within purified enzyme, Hat1 is associated with a second subunit, Hat2, which is required for strong binding to histone H4 and contributes to substrate specificity (185). Hat2 is a member of a protein family defined by RbAp48, a human retinoblastoma (Rb)-interacting protein that acts as an apparent histone H4 chaperone (198) and is also a subunit of human chromatin assembly factor CAF-1 (249) and histone deacetylase HDAC1 (235). In vitro, Hat1 enzyme can acetylate lysine-12 of the histone H4 N-terminal tail region (127, 185), previously identified as one of the major residues acetylated in newly synthesized histones (45, 218). Although Hat1 is thought to be deposition related, recent evidence suggests that it is not entirely cytoplasmic. Hat1 and Hat2 were found to be part of a nuclear HAT activity on free (but not nucleosomal) histones, indicating its potential involvement in chromatin assembly in a more direct manner, perhaps at replication forks or silenced telomeres (200). Furthermore, a recently characterized HAT complex from human S-phase nuclei contained homologs of Hat1 and Hat2 and had in vitro specificity similar to that of the yeast enzyme, suggesting conservation of its function throughout eukaryotes (248).

Elp3, a yeast A-type HAT, appears to have a direct role in transcription in that it is part of the RNA polymerase II holoenzyme and is involved in transcriptional elongation. In *S. cerevisiae*, the three-subunit elongator complex binds tightly to RNA polymerase II and its hyperphosphorylated C-terminal repeat domain (CTD), participating in an elongation-competent form of holoenzyme (182). Elp3, the smallest elongator subunit, was identified by peptide mass spectrometry and found to have GNAT homology (269). Genetic studies showed that an *elp3* null mutant was viable but displayed defective phenotypes similar to those of a previous elongator null mutant, *elp1* (182): slow activation of certain genes, slow growth adaptation, and salt and temperature sensitivity. Because of its GNAT homology, recombinant Elp3 was produced from insect cells and tested for HAT activity in in-gel assays. Under these conditions, Elp3 was able to acetylate all four core histones when presented with them individually (269). Although the specific function of this HAT activity and its in vivo role remain to be characterized, a clear model emerges, built on insight gained from studies on Gcn5. Since Gcn5's HAT activity is known to cause remodeling of promoter DNA (89) and is thought to assist transcriptional initiation, Elp3 may by analogy facilitate transcript elongation by modifying chromatin within a gene, thereby clearing the way for holoenzyme. Like Gcn5, Elp3 function may be redundant with other mechanisms, since its gene is not essential, but its possible importance is demonstrated by its evolutionary conservation in homologs from various other eukaryotes, including mammals (269).

Hpa2 is the most recently described HAT protein as of this writing, and only limited information has been published about it at this point. As a GNAT superfamily member, this yeast protein was tested in vitro and found to acetylate histones H3 and H4, with a preference for lysine-14 of H3, like the Gcn5 subgroup (5). Interestingly, Hpa2 has a high degree of homology with another yeast GNAT protein, Hpa3, which displayed very poor HAT activity in in vitro assays but did autoacetylate (as did Hpa2). Hpa2 can form a dimer or a tetramer in vitro, and the crystal structure of the tetramer has been determined (5). In vivo, however, Hpa2 has unknown function, as a knockout of the gene conferred no apparent growth phenotype. Further genetic and biochemical studies will be required to determine the roles of this protein and the potential HAT Hpa3 in the cell.

Structure and mechanism. Along with mutant studies of yeast *GCN5*, structure determination of several Gcn5-related proteins has added to our knowledge of the mechanisms of acetylation by these enzymes. The first two GNAT superfamily members to have the crystal structures of their acetyltransferase domains solved were yeast Hat1 (59) and *Serratia marcescens* aminoglycoside *N*-acetyltransferase (270), a bacterial enzyme that inactivates certain antibiotics by acetylation. In each case, a truncated, catalytically active fragment of the protein bound to CoA or acetyl-CoA substrate was crystallized. Subsequently, HAT domain structures from the Gcn5 subgroup—*Tetrahymena* (146, 197) and yeast (241) Gcn5 and human PCAF (48)—and HAT protein Hpa2 (5) were also determined.

The central regions of these six proteins all have very similar topologies, and together they define a fundamental structure for GNAT acetyltransferases. As shown by the example of the *Tetrahymena* Gcn5 HAT domain in Fig. 3, the proteins consist of N-terminal and C-terminal domains separated by a deep hydrophobic cleft. A conserved core, formed by a three-stranded β-sheet and an amphipathic α-helix and encompassing GNAT motifs A and D, lies at the bottom of the cleft. The acetyl-CoA substrate binds in part of the cleft and is held between motif A and motif B, which located in the C-terminal

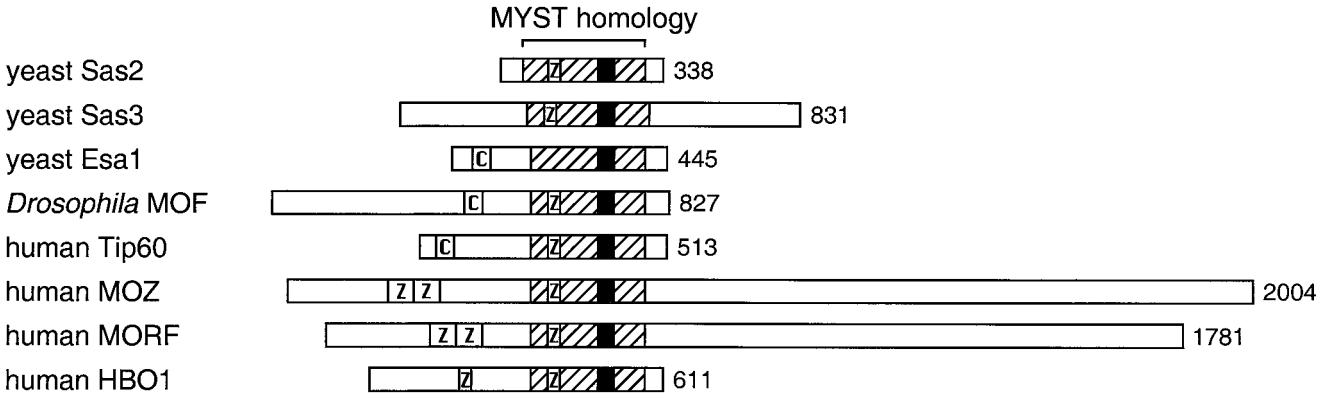

FIG. 4. Alignment of the MYST family of HATs and putative HAT proteins. The MYST homology region is indicated, with the acetyl-CoA-binding site, corresponding to GNAT family motif A, shown as a black box. Z, zinc finger motifs: an atypical C2HC motif in the MYST region (Esa1 diverges from this motif), a typical C2HC in the N-terminal region of HBO1, and two adjacent C4HC3 (or PHD) fingers in MOZ and MORF. C, chromo-like domain found in Esa1, MOF, and Tip60.

domain. One *Tetrahymena* Gcn5 study in particular (197) provided additional information about HAT function by presenting the structure of a ternary complex containing a histone H3 N-terminal tail peptide as well as the HAT domain and CoA. The histone peptide was shown to occupy the larger part of the cleft, bringing the side chain of acetylatable lysine-14 in proximity to CoA (Fig. 3).

The catalytic site and mechanism of histone acetylation by Gcn5 have also been defined as a result of the structure determinations and mutational analyses. Acidic residues within the cleft region of yeast Gcn5 were likely candidates to function as a general base for catalysis; of these, only glutamate-173 was conserved among the Gcn5/PCAF homologs and potentially critical for function, since simultaneous alanine substitution of glutamate-173 and phenylalanine-171 led to major defects (256). The position of *Tetrahymena* Gcn5's glutamate-122 (analogous to yeast glutamate-173) relative to the substrates is shown in Fig. 3. Further yeast studies used a mutant in which glutamate was replaced with glutamine (which has a similar side chain structure but no acidic group) and found that this mutant was highly defective for HAT activity in vitro (234) and for growth and transcription in vivo (241). It was therefore concluded that the carboxyl moiety of glutamate-173, by deprotonating the lysine substrate, is crucial for the HAT catalytic mechanism and overall function of Gcn5. Altogether, the structural, mutational, and GNAT conservation data were in close agreement for the Gcn5 proteins, also allowing detailed mapping of the substrate-binding determinants (residues critical for acetyl-CoA and histone interaction) (197). With regard to catalysis, however, it should be noted that the critical glutamate residue is only conserved among the direct Gcn5 homologs and PCAF but not the other GNAT HATs or other acetyltransferases. Therefore, in non-Gcn5 acetyltransferases, catalysis may occur through other side chains or by direct nucleophilic attack between the substrates (59).

While the studies described above have focused on GNAT catalytic domains, the bromodomain is another Gcn5 region for which there are structural and functional data suggesting an involvement in HAT function. The bromodomain (whose name is derived from Brahma, the *Drosophila* protein in which it was first described) (233) is a conserved sequence motif found in PCAF and the Gcn5 homologs as well as a variety of other transcription-related proteins (98). Its precise function is largely unclear, but it has been theorized to be involved in protein-protein interactions (117, 268). In vitro, the bromodomain is not required for recombinant yeast Gcn5 to acetylate free histones (37). However, bromodomain deletion of Gcn5 did cause partial defects in growth and in vivo transcription of certain genes (76, 154) and also resulted in reduced in vitro nucleosome acetylation in the context of a native complex, SAGA (described below) (223), suggesting that the bromodomain does have a HAT-related functional effect. Recent evidence indicates that this effect may involve histone interaction. In vitro binding studies demonstrated that the yeast Gcn5 bromodomain interacts directly with histone H3 and H4 N-terminal tails (181), and a structure determination of the PCAF bromodomain showed that it forms a four-helix bundle with a hydrophobic pocket that binds acetyl-lysine on histone H3 or H4 peptides (53). Together, these results suggest a potential role of HAT bromodomains in contributing to substrate interaction, in addition to possible tethering to chromosomal sites (30) or other protein interactions of a regulatory nature (11, 268).

MYST Family

Another group of evolutionarily related proteins that are known or hypothesized to be HATs is the MYST family, named for its founding members: MOZ, Ybf2/Sas3, Sas2, and Tip60 (23). Additional members have more recently been identified, including yeast Esa1, *Drosophila* MOF, and human HBO1 and MORF. These proteins are grouped together on the basis of their close sequence similarities (Fig. 4) and their possession of a particular acetyltransferase homology region (part of motif A of the GNAT superfamily) (174), as shown in Fig. 1A. Although containing regions similar in sequence, the members of the MYST family are involved in a wide range of regulatory functions in various organisms.

Sas2 and Sas3. One of the diverse functions mentioned above is transcriptional silencing, which in *S. cerevisiae* involves at least two MYST proteins, Sas2 and Sas3 (also known as Ybf2). The *SAS2* (something about silencing) gene was originally discovered in a screen for defects in epigenetic silencing in a *sir1* genetic background (194). *sir1* null mutation leads to loss of mating in most cells due to defects in silencing at the *HM* mating type loci, but a subpopulation of cells remain able to mate. Additional mutation of *SAS2*, however, led to absence of mating, even though a *sas2* single mutant was phenotypically normal. Interestingly, Sas2 seems to have opposite regulatory

effects depending on the silenced locus, promoting silencing at *HML* while inhibiting it at *HMR* (64). Other tests demonstrated that Sas2 was required for telomeric silencing (194). Sas3 is a second silencing-related yeast MYST protein, identified by its close homology to Sas2. A *sas3* single mutant was also phenotypically normal, and subsequent mutant studies showed that Sas3 has overall weaker effects than Sas2: it is involved in silencing at mating loci, since a *sas3* mutation (like *sas2*) restored silencing to a partially defective *HMR* locus but did not affect silencing at telomeres (194).

Sas3 is a confirmed HAT, as recent in vitro experiments have demonstrated that glutathione-*S*-transferase (GST)-fused Sas3 can acetylate free histones H3 and H4 strongly and H2A weakly (230). Furthermore, Sas3 is the catalytic subunit of the nucleosomal H3-acetylating complex NuA3, described below (S. John and J. L. Workman, unpublished results). Although HAT activity has not yet been demonstrated for Sas2 in vitro, it may require additional subunits or in vivo modifications in order to function enzymatically.

In vivo, chromatin structure is known to be highly important for transcriptional silencing, which correlates with reduced nucleosome acetylation (28). While negative effects on silencing (such as at the *HMR* locus) would fit with traditional models of histone acetylation, the positive silencing effects seen with these two potential HATs are suggestive of more complicated regulatory mechanisms. Alternatively, Sas2 or Sas3 may achieve regulation by acetylating substrates other than histones. This is possibly supported by findings that loss of yeast N-terminal acetyltransferase activities leads to silencing defects (7, 170, 264) and by the growing list of known factor acetyltransferases, discussed later in this review. However, discovery of specific silencing mechanisms will require future study.

Esa1. A third yeast MYST family protein, Esa1, has recently been identified and characterized as an essential HAT required for cell cycle progression. Esa1 was originally identified through its homology with Sas2, Sas3, and other MYST proteins, and a null mutant of its gene was inviable, hence its name (essential Sas family acetyltransferase 1) (216). Esa1 is a HAT, as recombinant protein was able to acetylate free histones H2A, H3, and H4 in vitro, with its strongest activity on histone H4, particularly at lysine-5. It was unable, however, to acetylate nucleosomes in vitro. In vivo, loss of Esa1 led to specific defects in histone acetylation and growth (47). When *esa1* temperature-sensitive mutants were grown at the restrictive temperature, the lysine-5-acetylated form of histone H4 was partially lost (extracts were probed with antibody specific to this isoform). Furthermore, flow cytometric and microscopic analyses of these mutants revealed that cells that lose Esa1 exhibit G_2/M arrest, blocked in the cell cycle subsequent to DNA replication but prior to mitosis and cell division (47). Taken together, these findings demonstrate the importance of the Esa1 protein in yeast cellular function, and its direct connection to transcription has recently been shown by studies with a native Esa1-containing complex, NuA4 (described below).

MOF. In *Drosophila melanogaster*, the MOF protein is a MYST family member with an important role in another transcriptional regulatory process, dosage compensation. Since male fruit flies have only one copy of the X chromosome compared to females' two, dosage compensation occurs in males to cause a twofold increase in the expression of X-linked genes (reviewed in reference 121). Association of a dosage compensation complex (123) with the chromosome is correlated with increased acetylation of histone H4 at a specific residue (lysine-16) (22, 245). The mechanism of this process was elucidated with the characterization of the *mof* (males absent on the first) mutation, which made male flies inviable. The gene product MOF was found to have MYST homology, and its direct link to histone acetylation was demonstrated by the fact that dying *mof* mutant males lack the lysine-16-acetylated isoform of histone H4 normally associated with the X chromosome (103). Interestingly, the mutation (mof^1) leading to nonfunctional MOF was a single glutamate substitution at a GNAT motif A invariant glycine residue implicated in acetyl-CoA substrate binding.

Recent studies with MOF and a native complex containing it (the MSL complex) have provided confirmation of MOF as a *Drosophila* HAT of histone H4 (217). In vitro, a recombinant fragment of MOF had an overall histone specificity similar to that of Esa1, acetylating H4 strongly and H2A and H3 weakly. Furthermore, partially purified MSL complex—containing MOF, several dosage compensation-specific proteins, and X chromosome-associated RNA—was able to acetylate nucleosomes specifically on lysine-16 of histone H4 in vitro. This activity was MOF dependent, as immunoprecipitated MSL complex containing mof^1-derived protein was essentially inactive (217). Altogether, the data are consistent with MOF's being the HAT responsible for a specific chromatin modification associated with dosage compensation.

Tip60. The first human MYST protein to be discovered, Tip60, also demonstrated a potential direct relationship between activation and histone acetylation. Tip60 (Tat-interactive protein, 60 kDa) was identified in a yeast two-hybrid/human library screen seeking proteins that interact with the activation domain of the HIV-1 transactivator protein Tat; specific physical interaction was further demonstrated by binding of expressed Tip60 to purified Tat in vitro (120). A recombinant construct of Tip60 lacking the N-terminal 40% but containing the MYST domain homology region was subsequently shown to have in vitro HAT activity, acetylating free histones H2A, H3, and H4 on specific lysines but acetylating nucleosomes poorly (125, 277). The findings of HAT activity and Tat interaction have recently provided insights into the cellular function of Tip60, as the Tat-repressed gene for Mn-dependent superoxide dismutase (262) was tested and found to be positively regulated by Tip60 in vivo. Furthermore, Tat was found to prevent this activation by specifically inhibiting the HAT activity of Tip60, leading to the hypothesis that Tip60 normally activates a set of genes by histone acetylation but that their expression can be opposed by Tat-mediated HAT inhibition (50). More information on the physiological functions of Tip60 may be provided by the very recent identification of a native, nucleosome-acetylating Tip60 complex, described later in this review (Y. Nakatani, unpublished results).

MOZ and MORF. While Tip60 is apparently associated with the action of HIV, MOZ is a MYST protein involved in another specific human disease process, oncogenic transformation leading to leukemia. When a particular chromosomal translocation in acute myeloid leukemia was characterized, it was found to have resulted in the fusion of two apparent HATs, the novel protein MOZ (monocytic leukemia zinc finger protein) (23) and CBP (described below). This created a chimeric protein consisting of the N-terminal three-quarters of MOZ (including its MYST and zinc finger domains) fused to the C-terminal 90% of CBP, containing its HAT domain and activator interaction regions. Although acetyltransferase activity of MOZ has not been directly demonstrated, it is hypothesized that MOZ-CBP may cause aberrant chromatin acetylation due to mistargeting of specific HAT activities, ultimately leading to leukemogenesis.

MOZ fusion with another transcription-related protein, TIF2, has also recently been reported in certain cases of leu-

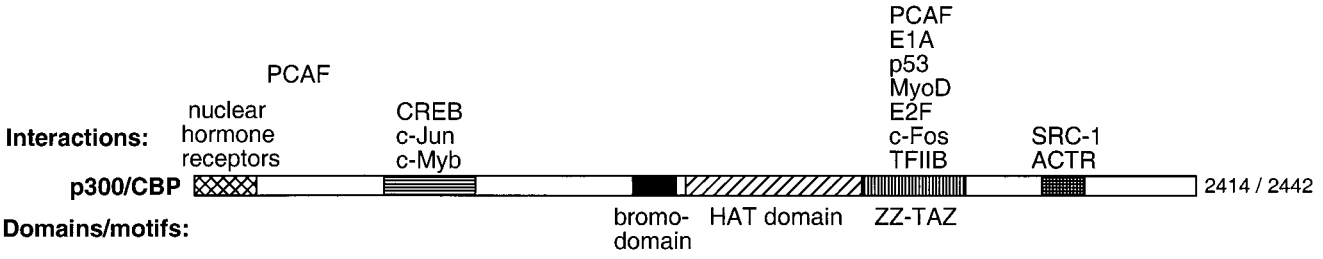

FIG. 5. Domains and interaction regions of the global coactivator HATs p300/CBP. Labeled below the polypeptide diagram are several domains and sequence motifs, including a bromodomain, the HAT domain, and ZZ and TAZ putative zinc fingers (190). Above are indicated some of the proteins demonstrated to interact with p300/CBP at certain regions. PCAF has been shown to interact with two regions of p300/CBP (130).

kemia (38, 144). These translocations also contained an N-terminal portion of MOZ, in this case fused to the C-terminal part of the nuclear receptor coactivator TIF2 (described further below), including its putative CBP interaction and activation domains. One hypothesis is that this fusion, through TIF2 interaction with CBP, may function similarly to MOZ-CBP, with equivalent aberrant effects. But interestingly, TIF2's own putative HAT domain (42) is part of the fusion, so another misdirection of HAT function may be at work instead. Further characterization of MOZ and TIF2 transcriptional and HAT activities will be required to elucidate their roles in leukemogenic processes.

Another human MYST family member is MORF (MOZ-related factor), which was identified in a database search by its sequence similarity to MOZ and has recently been characterized (41). MORF shows very close homology to MOZ throughout its length, not just in the MYST consensus region. Although MORF mutation has not yet been implicated in cancer, as MOZ has, its in vitro HAT function has been more thoroughly studied, perhaps shedding light on the function of both proteins. Recombinant full-length MORF expressed in insect cells and a bacterially produced MYST domain fragment were both able to acetylate free histones in vitro, with a preference for H3 and H4. Furthermore, the insect-derived protein was also competent for nucleosome acetylation, strongly preferring histone H4. Another finding was that MORF contains an N-terminal repression region (including two zinc fingers), deletion of which led to increased in vitro HAT activity and increased in vivo transcription by Gal4-MORF at a reporter gene. Interestingly, alternative forms of MORF (MORFα and MORFβ) have been observed which have insertions at a site within or near the repression domain, but their impact on MORF function is not yet known. In addition, MORF contains a C-terminal activation domain that is functional in the absence of the HAT domain; the analogous C-terminal region is missing in the MOZ translocations. While MOZ and MORF, like Gcn5 and PCAF, are very closely related in sequence, it remains to be determined how functionally similar they are and in which specific transcriptional processes they participate.

HBO1. A fourth human MYST protein is HBO1 (histone acetyltransferase bound to ORC), which was discovered in a two-hybrid screen on the basis of its interaction with the ORC1 subunit of the origin recognition complex (ORC) (112). ORC is conserved throughout the eukaryotes and is primarily known to bind DNA replication origins and to be critical for the initiation of replication (13, 60). ORC also has a transcriptional function, however, since it has been demonstrated to be involved in silencing at yeast mating type loci (12, 69, 71) and *Drosophila* heterochromatin regions (184). In the case of *S. cerevisiae*, a relationship with the MYST proteins Sas2 and Sas3 is suggested by the fact that ORC binds Sir1 (70, 242) and that Sas2 displays genetic interactions with ORC (*SAS2* knockout results in partial suppression of *orc2* and *orc5* mutant phenotypes) and antagonizes ORC-mediated silencing at the *HMR* locus (64).

Upon the cloning of HBO1 and discovery of its MYST homology, its HAT function was investigated. Via HBO1-specific antibodies, an HBO1-containing complex was isolated from nuclear extract and found to acetylate free histones H3 and H4 well and nucleosomes weakly. Recombinant HBO1 alone was not observed to acetylate free histones, but it did exhibit some HAT activity, as very weak acetylation of nucleosomal histones was seen (112). Full activity of the HBO1 protein may therefore require other factors or in vivo modifications. The in vivo function of HBO1 and its role in transcriptional silencing remain to be studied, and its relationship to the yeast Sas proteins is still unknown. While a logical hypothesis is that HBO1 may be a functional analog of one of these proteins, none of them (Sas2, Sas3, or Esa1) bound directly to yeast Orc1 in a two-hybrid assay (112).

p300/CBP

After the discovery of histone acetylation by Gcn5 and PCAF, the critical role of acetyltransferases in transcriptional regulation was also demonstrated by the fact that a pair of previously well-characterized coactivators of multicellular eukaryotes, p300 and its close homolog CBP (CREB-binding protein), are themselves HATs (8, 178) and FATs (as described below). The interactions of p300/CBP (p300 and CBP are often referred to as a single entity, since the two proteins are considered structural and functional homologs) with PCAF and GCN5, described above, and with nuclear receptor coactivators, described below, are examples of transcriptional regulatory complexes with multiple acetyltransferase activities.

p300/CBP is a ubiquitously expressed, global transcriptional coactivator that has critical roles in a wide variety of cellular processes, including cell cycle control, differentiation, and apoptosis (81, 211), and mutations in p300 and CBP are associated with certain cancers and other human disease processes (80). On the molecular level, p300/CBP stimulates transcription of specific genes by interacting, either directly or through cofactors, with numerous promoter-binding transcription factors such as CREB, nuclear hormone receptors, and oncoprotein-related activators such as c-Fos, c-Jun, and c-Myb. As described above, p300/CBP also binds the HAT PCAF, an interaction with which adenoviral oncoprotein E1A competes (279). p300/CBP is a large protein of about 300 kDa and more than 2,400 residues, and at least four interaction domains with different sets of factors have been characterized throughout its sequence, as shown in Fig. 5. Furthermore, its central region

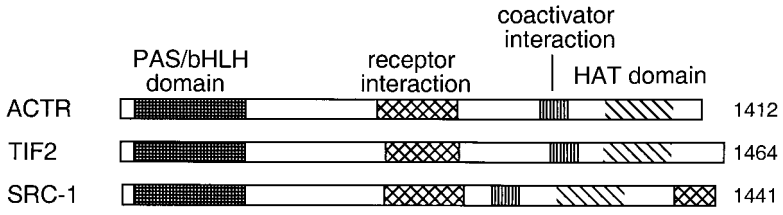

FIG. 6. Alignment of the p160 family of mammalian nuclear receptor coactivators. Indicated are the PAS/basic helix-loop-helix homology (bHLH) domain, nuclear receptor interaction regions, and the general area of interaction for coactivators p300/CBP and PCAF. ACTR and SRC-1 each have a HAT domain near their C terminus (42, 220), although the boundaries of these domains have only been approximately defined, and TIF2's domain is inferred by homology.

contains a bromodomain motif (98, 117), which is also found in the HATs Gcn5, PCAF, and $TAF_{II}250$.

The HAT activity of p300/CBP was first discovered in an E1A pulldown from HeLa (human) nuclear extract (178) and in direct CBP immunoprecipitations from Cos (primate) cell extracts (8). In vitro studies with recombinant p300 and CBP proteins confirmed that these proteins were indeed HATs, strongly acetylating the amino-terminal tails of all four core histones with little apparent specificity. Unlike other HATs, recombinant p300/CBP was able to acetylate all four histones within nucleosomes as well as in free-histone form. Deletion mutant analysis mapped the HAT domain of p300/CBP to an interior region between the bromodomain and the PCAF/E1A/MyoD/c-Fos interaction region (8, 178). p300/CBP represents a unique class of acetyltransferase, although it may be distantly related to other HATs. Careful sequence analysis identified regions with limited homology to GNAT motifs A, B, and D, in addition to another short motif shared with PCAF and Gcn5 (158). Site-directed mutagenesis demonstrated that all four of these motifs contribute to CBP's HAT function. Furthermore, the connection between p300/CBP's HAT function and transcription in vivo was demonstrated by the fact that a promoter-tethered CBP HAT domain resulted in activation, and HAT-impaired mutant versions showed a direct correlation of acetylation competence with this transcriptional activity (158). p300/CBP's HAT function was also shown to be required for certain types of nuclear receptor-mediated activation in vivo (130).

In addition, the HAT activity of p300/CBP is apparently regulated by other factors. As observed for PCAF, the viral protein E1A and the regulatory protein Twist were shown to bind to p300 and inhibit its HAT activity (40, 96, 187). However, another report indicates that E1A has a HAT-stimulatory effect on CBP (2), suggesting a possible functional difference between p300 and CBP (this study also found that cell cycle-dependent phosphorylation of CBP by Cdk2 increases its HAT activity). Since another study reported no effect of E1A binding on CBP's HAT activity (8), it is possible that these HAT effects are due to experimental discrepancies that need to be resolved.

Overall, p300/CBP is one of the most potent and versatile of the acetyltransferases, consistent with its role as a global coactivator in higher eukaryotes. Like PCAF, p300/CBP is known to acetylate and regulate various transcription-related proteins other than histones. The known FAT substrates of p300/CBP, described later in this review, include HMG I(Y), activators p53, GATA-1, erythroid Krüppel-like factor (EKLF), *Drosophila* T-cell factor (dTCF), and HIV Tat, nuclear receptor coactivators SRC-1, ACTR, and TIF2, and general factors TFIIE and TFIIF. Another phenomenon relevant to the regulatory activities of p300/CBP is that human chromosomal translocations fusing CBP to either the putative HAT MOZ (23) or the MLL gene (232) can result in leukemogenesis; the mechanisms of these processes, however, and whether they involve HAT or FAT activity remain to be elucidated.

Nuclear Receptor Coactivators

HAT proteins have also been directly implicated in transcriptional activation brought about by hormone signals. The HAT activities of human coactivators ACTR and SRC-1, which interact with nuclear hormone receptors, demonstrate the involvement of acetylation in yet another system of transcriptional regulation and define a unique family of HATs.

SRC-1. Steroid receptor coactivator-1 (SRC-1), also known as p160 (119) and NCoA-1 in mice (240), is a human nuclear receptor cofactor originally discovered by way of its interaction with the human progesterone receptor (PR) in a yeast two-hybrid screen. In vivo experiments in mammalian cells established the coactivator function of SRC-1, as it was able to stimulate ligand-dependent activation by numerous nuclear receptors, including PR, glucocorticoid receptor (GR), estrogen receptor (ER), thyroid hormone receptor (TR), and retinoid X receptor (RXR) (180). Because of this coactivator function, recombinant SRC-1 was assayed in vitro and found to have HAT activity, acetylating H3 and H4 either as free histones or in mononucleosomes (220). Truncation analysis revealed that the HAT domain is located in the C-terminal region of SRC-1, as diagrammed in Fig. 6. SRC-1 was known to interact with p300/CBP (119, 214, 280), and interestingly, it also interacted with PCAF in vitro and in vivo (220), indicating that multiple HATs are employed to regulate hormone-signaled transcription. In addition, p300/CBP was recently shown to acetylate SRC-1, an event that is likely relevant to its nuclear receptor coactivator function (43).

ACTR. To identify additional human proteins that interact with nuclear hormone receptors, a yeast one-hybrid screen was employed which used reporter genes with retinoic response elements and a human retinoic acid receptor (RARβ) as bait. Screening with a cDNA library resulted in several known receptor interactors (including SRC-1) and one novel cofactor, termed ACTR (42), also known as RAC3 (142), AIB1 (6), and TRAM-1 (231) in humans and p/CIP in mice (240). Like SRC-1, ACTR was shown to interact with multiple nuclear hormone receptors and stimulate transactivation. Further, it was tested in vitro and also found to be a HAT capable of acetylating free or nucleosomal histones H3 and H4, and its HAT domain similarly mapped to the C-terminal end of the protein (42). In fact, ACTR shows significant sequence similarity to SRC-1 in several regions (Fig. 6): an N-terminal, basic helix-loop-helix/PAS region (236), receptor and coactivator interaction domains, and the C-terminal HAT region, defining, along with TIF2, the p160 (or SRC) family of nuclear receptor coactivators (42, 139, 252).

Further similarities between ACTR and SRC-1 are their interaction with CBP and PCAF and their acetylation by CBP. Acetylation of ACTR has been more thoroughly characterized, and it has distinct functional effects. Specifically, the acetylation occurs in the receptor interaction domain, preventing receptor binding and hence activation by ACTR (43). ACTR is therefore both a HAT and a regulatory target for another acetyltransferase.

TIF2. A third potential HAT in the human nuclear receptor coactivator family is TIF2 (transcriptional intermediary factor 2) (252), also known as GRIP1 (106) and NCoA-2 (240) in mice. Like SRC-1 and ACTR, TIF2 binds to a number of nuclear hormone receptors, stimulates transcriptional activation (252), and interacts with (251) and is acetylated by (43) CBP. Although its HAT activity has not yet been demonstrated, TIF2 has all of the homology regions shared by SRC-1 and ACTR, including the putative HAT domain (42). Because of the sequence and functional similarities of this protein to the other two coactivators, it stands as a likely HAT candidate whose activity remains to be characterized. Another potentially interesting aspect of TIF2 is its fusion to MOZ in leukemia-associated translocations, as noted above (38, 144). Future studies will be required to determine the mechanism of this oncogenic effect and whether it involves either putative HAT activity.

The three nuclear receptor coactivators discussed above are part of an evolutionarily and functionally related HAT family; all three interact with p300/CBP, and at least two interact with PCAF. However, recent studies have demonstrated that p300/CBP (119) and PCAF (21, 130) can directly interact with nuclear receptors, independent of other factors. Furthermore, the MYST family protein Tip60 was also recently discovered to function as a coactivator with several receptors in a ligand-dependent manner (26). The fact that p300/CBP, PCAF, and Tip60 can also function as nuclear receptor coactivators underscores the importance of acetylation in transcriptional response to hormone signals and demonstrates that in higher eukaryotes, multiple strategies of acetyltransferase recruitment are used for this process.

TBP-Associated Factor $TAF_{II}250$

Another direct connection between acetylation and activated transcription was demonstrated with the discovery that one of the TAF_{II} (TATA-binding protein [TBP]-associated factor) subunits of the general transcription factor TFIID is itself a HAT. Specifically, homologs of this protein—$TAF_{II}250$ in humans, $TAF_{II}230$ in *Drosophila*, and $Taf_{II}145/130$ in *S. cerevisiae*—were shown to have HAT activity in vitro (169).

TFIID is one of the general factors required for the assembly of the RNA polymerase II transcription preinitiation complex, along with TFIIA, TFIIB, TFIIE, and TFIIF (32, 97). TFIID is in fact the first factor needed in the stepwise assembly: through its TBP subunit, TFIID binds to specific promoter DNA sequences and allows subsequent formation of the transcription complex. Although TBP without TAF_{II}s is able to bind promoters and allow basal transcription in vitro, the TAF_{II} subunits promote activated transcription. Furthermore, TAF_{II}s have been shown to interact with certain activators and initiation-related factors (250).

The potential involvement of acetylation in TAF_{II} function was realized with the discovery that a 250-kDa band from human nuclear extract (in an in-gel assay) and immunoprecipitated human TFIID had HAT activity (169). Further characterization of the TAF_{II} HAT activity was performed with recombinant *Drosophila* $TAF_{II}230$, which was found to acetylate H3 (preferentially on lysine-14, like Gcn5) and H4 in a free histone mixture (and H2A as an individual histone). It should be noted that $TAF_{II}250$ and its homologs, like the p160 nuclear receptor coactivators, have some of the weaker in vitro HAT activities observed—p300/CBP and PCAF, for example, have more potent activities (130, 177; unpublished results). The in vivo significance of these apparent differences in catalytic strength, however, is not yet known.

Truncation studies with yeast and *Drosophila* TAF mapped the HAT domain to the conserved central region of the protein. This region has little apparent similarity to other known proteins, so $TAF_{II}250$ may define a unique HAT class. However, a potential acetyl-CoA binding site has been identified within this region; it shares a Gly-X-Gly pattern with Gcn5 and other acetyltransferases, and mutation of these glycines led to reduced HAT activity (58). Like Gcn5, PCAF, and p300/CBP, $TAF_{II}250$ also has a bromodomain (and *Drosophila* $TAF_{II}230$ has two), but truncation studies demonstrated that it is not required for HAT activity (169); this and the fact that the yeast homolog contains no bromodomain argue against a major role for it in $TAF_{II}250$'s HAT function.

The HAT activity of $TAF_{II}250$ and its homologs suggests a model for the initiation of transcription complex formation at chromatin-packaged promoters. Nucleosomes are known to inhibit binding of TBP to the TATA box (164, 273), and this inhibition is apparently mediated by histone tails (82, 115). As part of TFIID, $TAF_{II}250$ may well facilitate TBP binding directly by acetylating histones at the TATA box, allowing formation of the preinitiation complex. Also potentially relevant to $TAF_{II}250$ function is that TFIID is proposed to contain a histone octamer-like structure (104, 274), which may displace nucleosomal histones in concert with $TAF_{II}250$'s HAT activity. Although the widespread involvement of TFIID in initiation (including at TATA-less promoters) is expected to bring $TAF_{II}250$ to very many genes, recent mutant studies suggest that its HAT activity is required for transcription at only a subset of promoters (e.g., certain cell cycle regulators) (58, 176). The mechanism of this specificity, however, is not yet known.

TFIIIC

Although all of the A-type HATs discussed so far in this review are proposed to be involved with transcription by RNA polymerase II (primarily of mRNA), chromatin structure is expected to affect any kind of transcription, such as the synthesis of rRNA by RNA polymerase I or tRNA precursors by RNA polymerase III. Evidence that histone acetylation is a generally employed mechanism in transcription is the fact that subunits of TFIIIC, a general transcription factor in the RNA polymerase III basal machinery, were also recently identified as HATs (109, 133). The known function of TFIIIC is to initiate transcription complex formation by binding to promoter DNA and recruiting TBP-containing TFIIIB and RNA polymerase III (137). Recent in vitro studies with purified human TFIIIC showed that it harbored HAT activity, acetylating H3, H4, and H2A as free histones and also in nucleosomes. Interestingly, an in-gel assay of TFIIIC revealed that three of its nine subunits have apparent HAT activity. The HAT functions of two of these subunits, TFIIIC110 and TFIIIC90, have been confirmed and further investigated. A bacterially expressed C-terminal fragment of TFIIIC110 had HAT activity in an in-gel assay (133), while recombinant TFIIIC90 was competent for the acetylation of either nucleosomal or free histone H3, with an apparent preference for lysine-14 (like Gcn5 and $TAF_{II}230$) (109). Future studies should better clarify the function of these HAT activities in this

type of transcription, but a logical hypothesis is that it fulfills a role similar to that of $TAF_{II}250$ in the RNA polymerase II transcription complex. In both cases, a HAT enzyme is intimately associated with the first step in DNA binding of the transcription complex and likely acts to destabilize promoters' nucleosomes to facilitate this process. Furthermore, it is reasonable to predict that RNA polymerase I transcription is also associated with HAT activity, although this has not yet been demonstrated.

NUCLEOSOME-ACETYLATING NATIVE COMPLEXES

To participate in transcription in vivo, the HATs described above have often intricate interactions with various regulatory proteins and/or the transcription apparatus. These interactions can potentiate a HAT enzyme's activity at a particular genetic locus or time (i.e., cell cycle or developmental stage) or modulate substrate specificity—its choice of specific lysine residues in particular histone tails (H2A, H2B, H3, or H4) in a nucleosomal context—to bring about an appropriate transcriptional effect. Some of the native complexes containing HATs have been isolated and studied, and they rae described below.

Yeast HAT Complexes

Most known HATs are able to acetylate free histones in vitro when assayed as a single polypeptide. Many, however, such as Gcn5, are unable to acetylate their probable physiological substrate, nucleosomal histones, under standard conditions in vitro, apparently due to the requirement for other factors to allow this level of substrate specificity. Because of this, a study was performed which sought to identify native yeast complexes capable of acetylating nucleosomal substrates (84). Through fractionation of *S. cerevisiae* extracts and assays of nucleosomal HAT activity, four distinct complexes were discovered and have been further characterized: SAGA, ADA, NuA4, and NuA3.

SAGA. After their discovery, the four separable nucleosomal HAT activities were initially analyzed by Western blot and null mutation studies, and it was found that the two nucleosomal histone H3/H2B-specific complexes contained Gcn5 as their HAT catalytic subunit, along with two other transcriptional adaptor proteins, Ada2 and Ada3 (84). Interestingly, one of these complexes also contained several Spt proteins, which were originally identified via another transcription-related genetic screen (suppression of Ty and δ insertions at promoters) (reviewed in reference 267). This complex was therefore named SAGA (Spt-Ada-Gcn5 acetyltransferase) (reviewed in reference 88); the other complex, containing Ada proteins but not Spts, was called ADA (described below).

Of the known HAT-containing complexes, yeast SAGA is the best characterized. It is a large complex, approximately 1.8 MDa, as determined by a sizing column. About 15 of its subunits are now known, although it is expected that at least several more remain to be identified. Notably, SAGA brings together in one complex four different groups of previously described transcription-related proteins: the transcriptional adaptors (Ada proteins), a subset of the Spt proteins, a subset of the Taf_{II}s (86), and Tra1 (87, 204), the yeast homolog of the human transcriptional regulatory protein TRRAP. Interestingly, human HAT complexes have also been isolated that contain homologs of each of these groups, as shown in Fig. 7 and discussed below, suggesting evolutionary conservation of SAGA function (177, 247). In addition, yeast SAGA contains the transcriptional regulator Sin4 (282), which is also a component of the Srb/mediator subcomplex of RNA polymerase II holoenzyme (143, 219).

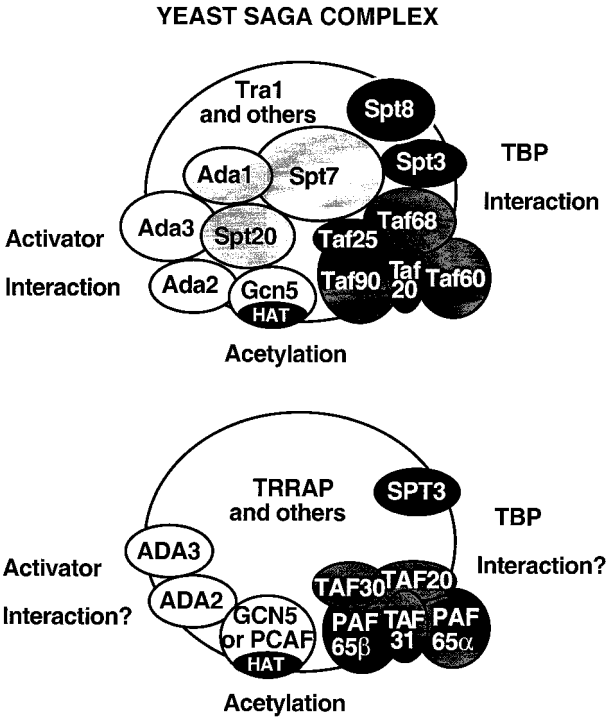

FIG. 7. Schematic diagram of known subunits and functions of the yeast SAGA and human PCAF or GCN5 HAT complexes. The yeast SAGA complex (top) contains Gcn5 as its HAT catalytic subunit. SAGA has been shown to interact with acidic activation domains, and this function may be mediated by its adaptor components (Ada2, Ada3, and Gcn5) or possibly through Tra1 or other subunits. Another subset of SAGA proteins (Ada1, Spt7, and Spt20/Ada5) are required for its structural integrity and overall function. The Spt3 and Spt8 subunits have been implicated in interaction with TBP, and the Taf_{II} group, which also participate in TFIID, may provide TBP-binding function to SAGA as well. The human SAGA-analogous complexes (bottom) contain either GCN5 or PCAF. The PCAF complex has been more thoroughly studied; it has nucleosome acetylation function, and known subunits include TRRAP, hADA2, hADA3, hSPT3, and TAF_{II}s/PAFs (PCAF-associated factors)—all homologs of proteins found in SAGA. Activator and TBP interaction functions are hypothesized for these human complexes but have not yet been demonstrated.

The adaptors contained in yeast SAGA are Ada1 (107a), Ada2 (17), Ada3 107b(), Ada5, and Gcn5. The Spt proteins it contains are Spt3 (64a), Spt7 (73), Spt8 (64b), and Spt20, previously described as the TBP-related Spt subgroup because of their apparent functional interactions with TBP. Prior to the discovery of SAGA, there was evidence of an Ada-Spt relationship in that *ADA5* and *SPT20* represent the same gene, which was discovered in independent genetic screens (153, 196). More recently, analysis of SAGA subunit mutant effects on phenotypes and on SAGA composition and function have shown that the Ada and Spt proteins within SAGA can be placed in three categories reflecting their structural and functional roles in the complex (84, 195, 223). Null mutation of the genes encoding Ada1, Spt7, or Spt20/Ada5 leads to disruption of SAGA and severely impaired growth, indicating the requirement of these subunits for SAGA structural integrity and the significant impact of SAGA loss in vivo. Mutations in either of the other two groups, Ada2/Ada3/Gcn5 and Spt3/Spt8, led to largely intact SAGA and moderately impaired yet distinct phenotypes, consistent with their roles as somewhat peripheral subunits that are involved in specific SAGA subfunctions that have been demonstrated—activator interaction (113, 246) and

nucleosome acetylation for Ada2/Ada3/Gcn5 and TBP interaction (15, 86, 195, 223) for Spt3/Spt8.

In vivo and in vitro, the SAGA complex and its components have been shown to be critical to certain types of transcription. In vitro, purified SAGA was able to stimulate transcription in various chromatin-template assays by way of its combined HAT activity and interaction with acidic activators (113, 246, 254). The in vivo significance of SAGA has been demonstrated by examination of mutants of its components, which have verified that the complex has an important role in transcriptional activation at a subset of genes, such as *GAL1* (57), *TRP3*, and *HIS3* (15), although its regulatory effect may be distinct at different genes. Interestingly, Gcn5/SAGA and the chromatin-remodeling complex Swi-Snf display apparent genetic interactions (189, 195) and complementarity or partial redundancy with each other in the activation of some genes (19, 90, 229). This suggests that both of these complexes may be recruited to certain promoters and contribute to transcriptional activation by altering chromatin, albeit by different mechanisms (14).

Likely relevant to the in vivo chromatin-modifying function of Gcn5 is the fact that its participation in the SAGA complex has distinct consequences for its histone substrate specificity in vitro. SAGA gives Gcn5 the ability to acetylate nucleosomes, with a primary specificity for histone H3 and, to a lesser extent, H2B (84). This capacity to interact with and recognize nucleosomal histones is apparently conferred by other subunits in the complex and may involve Gcn5's bromodomain, deletion of which significantly reduces nucleosome acetylation by SAGA (223). Participation of Gcn5 in the SAGA complex (and ADA) also causes expanded lysine specificity on histone H3, as determined by a recent study (85). SAGA and ADA significantly acetylated other lysine residues in addition lysine-14 both on H3 N-terminal tail peptides and in nucleosomal H3. The patterns of acetylation by these complexes were overlapping yet distinct, further indicating the influence of other subunits on Gcn5's function.

Future studies should further elucidate the roles of various subunits in the structure and transcriptional function of SAGA. It is notable that SAGA does not contain $Taf_{II}145/130$, the Taf_{II} shown previously to possess HAT activity (169), but it does contain a histone-related Taf_{II} subgroup ($Taf_{II}20$, -25, -60, -68, and -90), which is important for SAGA's acetylation and transcription-stimulation function in vitro (86). These subunits could conceivably provide TBP interaction or histone displacement function, but their specific roles in the context of SAGA remain to be demonstrated. Tra1, the yeast TRRAP homolog, also has implications for SAGA structure and function that require further study. Tra1 is an essential protein (204), and its large size (approximately 400 kDa) suggests that it may be important to the overall structure of SAGA. Functionally, its homolog TRRAP has coactivator function, interacting with the activators c-Myc and E2F (161), which suggests that it may have an activation domain interaction role like Ada2 (10, 213). Finally, recent evidence indicates that SAGA's composition and function may be dynamic, exhibiting changes depending on conditions in the cell. While SAGA produced from rich medium (transcriptionally repressive for *HIS3* and other amino acid-biosynthetic genes) has been well described, derepressing conditions gave rise to another form, termed $SAGA_{alt}$ (altered SAGA) (15). $SAGA_{alt}$ lacks the Spt8 subunit and, potentially, its negative regulation of TBP function at *HIS3*, but this complex and its precise relationship to SAGA await further characterization.

ADA. The other known Gcn5-containing complex is ADA, which has a size of about 800 kDa. Like SAGA, the ADA complex acetylates nucleosomes primarily on histones H3 and H2B in vitro, and it contains Ada2 and Ada3 but none of the other known subunits of SAGA (84). Recently, peptide analysis revealed a novel subunit unique to ADA, demonstrating that it is a distinct complex and not a subcomplex or artifactual fragment of SAGA (63). This subunit, Ahc1 (ADA HAT complex component 1), is required for the structural integrity of ADA, as a knockout mutation disrupted the complex.

Although ADA does contain Gcn5 and two other adaptors, unlike SAGA it does not seem to participate directly in transcription or have a major functional impact in vivo. Despite its possession of Ada2, a known interactor with acidic activators, ADA could not interact with activation domains in vitro, whereas SAGA could (246). Another functional difference between ADA and SAGA was demonstrated in their histone H3 lysine specificities in vitro; ADA acetylated fewer residues (lysine-14 and -18) than SAGA (lysine-9, -14, -18, and -23) (62, 85). Furthermore, an *ahc1*Δ mutation had no obvious phenotypic effects; the mutant (lacking the ADA complex) grew as well as wild-type cells on minimal medium and did not display an Ada^- phenotype or defects in in vivo transcription of a reporter gene (63). The physiological function of the ADA complex is still unknown, although some connection to histone acetylation in vivo has been suggested by the fact that overexpression of Ahc1 suppresses certain mutations in the gene encoding histone H2A (63).

NuA4. Another yeast HAT complex identified by Grant et al. (complex 2) was immediately distinguishable from the others in that its nucleosomal substrate was primarily histone H4 (as well as H2A, to a lesser degree) and it did not significantly acetylate histone H3 (84). Further purification and characterization of this 1.3-MDa complex, called NuA4 (nucleosomal acetyltransferase of histone H4), has revealed that its HAT catalytic subunit is the MYST protein Esa1 (3). It also contained Tra1, identified previously as a component of SAGA. Also like SAGA, NuA4 interacted with acidic activation domains in vitro and stimulated transcription in an acetylation-dependent manner in various in vitro assays with chromatin templates (3, 113, 246, 254). Interestingly, extensive acetylation of nucleosomal templates with NuA4 led to transcriptional activation even with other types of activators that do not interact with NuA4, an effect not seen with SAGA (113). This general activation by histone H4/H2A—as opposed to H3/H2B—acetylation shows the potential impact of nucleosomal histone specificity on transcription. NuA4's composition (it contains at least seven additional unknown subunits) and in vivo function remain to be fully characterized, but its possession of two essential transcription-related subunits, including a HAT needed for cell cycle progression (47), suggests that it plays a critical role in the cell.

NuA3. A fourth yeast HAT complex that has been identified and further investigated is NuA3 (also referred to as complex 3), a 500-kDa complex that exclusively acetylates histone H3 in nucleosomes (84). This is perhaps the least well characterized complex in terms of composition, but its catalytic subunit was recently determined to be Sas3, a MYST protein involved in silencing (S. John and J. L. Workman, unpublished results). Some in vitro studies have been performed with NuA3, and like ADA, it failed to interact with activation domains or to activate transcription in a specific way (246, 254). The function of this complex in vivo—i.e., its role, if any, in transcription or its relationship to silencing—remains to be determined by future studies.

Other complexes. Several other yeast complexes with HAT subunits and/or activity have also been discovered but await further characterization. For example, four complexes containing Ada2 and Ada3 (and, by inference, Gcn5) were recovered

from yeast extracts (203); of these, two approximately 2-MDa complexes were apparently SAGA, $SAGA_{alt}$, or related complexes (204). Another 900-kDa complex may be ADA, but the composition and function of a 200-kDa complex, and whether it contains Gcn5 and physiological HAT activity, remain to be determined. A separate study also identified three Gcn5-dependent activities that require further characterization (189). Another HAT-containing (Elp3) complex is elongator (269), but its HAT activity in the context of free elongator or RNA polymerase II holoenzyme has not yet been studied. Finally, there are certain remaining yeast HATs and putative HATs, such as Hpa2 and Sas2, for which no native complex has yet been identified. The nature of these unknown complexes, alternative complexes containing other HATs, and their enzymatic and possible transcription-related functions in vitro and in vivo are likely topics for future investigations.

Human HAT Complexes

Recently, several human protein complexes with known HAT subunits have been isolated from nuclear extracts and partially characterized. Subunit identification has shown that some of these complexes are remarkably analogous in composition to known yeast HAT complexes, and in each case an involvement in transcription is also suggested by subunits besides the HAT protein.

GCN5/PCAF complexes. One pair of human complexes was identified by way of N-terminal Flag epitope-tagged PCAF and GCN5, which were purified from HeLa nuclear extracts along with their native complexes to near homogeneity with a combination of conventional and antibody affinity chromatographies (177). Interestingly, when both complexes were analyzed on a Coomassie-stained SDS-PAGE gel to visualize all subunits, the patterns of bands were virtually identical, suggesting that the two complexes are very similar with the exception of the identity of the HAT subunit. Two of the GCN5 complex's subunits were confirmed immunochemically to be the same as those in the PCAF complex, further supporting the overall equivalence of the complexes. Since the GCN5 complex purification used the short form of GCN5, lacking the N-terminal 361 amino acids, these results imply that this region is dispensable for complex formation.

Of the two complexes, the PCAF complex has been more thoroughly characterized. It contained more than 20 polypeptides, and a subset of these have been identified by protein sequencing (177, 247). Remarkably, all 11 of the subunits identified so far are apparent homologs of components of yeast SAGA, suggesting strong evolutionary conservation of this type of complex (Fig. 7). Besides the Gcn5 homolog PCAF, the complex contained human adaptor homologs hADA2 and hADA3, Spt protein hSPT3 (281), the transcriptional cofactor TRRAP (161), and a set of five TAF_{II} or TAF_{II}-related proteins. Altogether, the identities of these subunits, like SAGA's, imply a transcriptional role for the PCAF complex (and, by analogy, the GCN5 complex), such as adaptor (through hADA2 and hADA3) and TBP interaction (through hSPT3) function. The c-Myc- and E2F-interacting subunit TRRAP, a member of the ATM superfamily (161, 247), further suggests a coactivator or other transcription-related role for these complexes.

The subunits shared between the PCAF complex and TFIID are $TAF_{II}20/15$, -30, and -31, homologs of yeast SAGA subunits $Taf_{II}68$, -25, and -17, respectively. Although the PCAF complex did not contain the TFIID-specific human homologs of yeast $Taf_{II}60$ and -90 (the other two Taf_{II}s in SAGA), it did contain two closely related proteins, termed PAF65α and PAF65β (PCAF-associated factors) (177). As in *S. cerevisiae*, most of these are histone-like TAF_{II}s, possibly suggesting the formation of a histone octamer-like substructure (274) which could displace nucleosomal histones during remodeling (177). Interestingly, Spt3, its homologs, and related $TAF_{II}18$ have been found to contain histone fold motifs (20), further suggesting structural parallels between TFIID, the SAGA/PCAF complexes, and the histone octamer (228).

As observed for Gcn5 in yeast SAGA, the participation of PCAF in this multisubunit complex has an effect on its HAT activity and specificity. Although recombinant PCAF can acetylate nucleosomal histones, primarily on histone H3, the PCAF complex acetylates H3 much more strongly (177). Like Gcn5, PCAF seems to require the influence of other subunits in a native complex to bring about its maximal activity on the more physiological substrate.

Two other human complexes, purified on the basis of TAF subunits, are apparently very similar to the human GCN5 complex. One complex, TFTC (TBP-free TAF_{II}-containing complex), was isolated from a nuclear extract by affinity purification with anti-$TAF_{II}30$ antibodies followed by immunodepletion of TBP (265). Like the PCAF complex, TFTC lacked TBP but contained a subset of the TAF_{II} proteins. Interestingly, this complex was able to support transcription in vitro from both TATA-containing and TATA-less promoters, functionally replacing TFIID despite the absence of TBP. The identity of TFTC as a HAT-containing complex with many of the same subunits as the GCN5 and PCAF complexes was revealed by a subsequent study (27), which demonstrated that TFTC can acetylate free and nucleosomal histones (with a preference for H3) in addition to linker histone H1. A number of TFTC's subunits were identified immunochemically, and it was found to contain the PCAF/GCN5 complex subunits GCN5, hADA3, hSPT3, PAF65β, and, of course, $TAF_{II}30$. However, TFTC contained several TAF_{II}s that the PCAF/GCN5 complexes apparently do not—$TAF_{II}150$, $TAF_{II}135$, and $TAF_{II}100$ (substoichiometric in the PCAF complex)—and did not contain human ADA2, suggesting that the complexes are not completely identical. The functional and in vivo significance of these differences is not yet known.

The other TAF_{II}-derived complex is STAGA (SPT3-$TAF_{II}31$-GCN5-L acetyltransferase), which was purified via antibodies specific for another histone H3-like TAF_{II}, $TAF_{II}31$ (157). The $TAF_{II}31$ immunopurified fraction was found by Western blot analysis to possess TBP and $TAF_{II}31$, as expected, as well as hSPT3 (281), the human homolog of yeast Spt3. TBP-containing species were eliminated by further affinity purification of the fraction with anti-hSPT3 antibodies, and analysis of the resulting complex showed that it also had HAT activity and contained GCN5. To date, hSPT3, $TAF_{II}31$, and GCN5 are the only known subunits of STAGA, and all three are evidently shared with both TFTC and the GCN5 complex, so further characterization will be required to discover STAGA's structural and functional similarity with each of these two slightly distinct complexes.

Tip60 complex. Very recently, a human complex containing another type of HAT, the MYST protein Tip60, has been purified and partially characterized (Y. Nakatani, unpublished results). This complex was isolated on the basis of N-terminal epitope-tagged Tip60, and it contained about 12 subunits, ranging from 29 to 400 kDa. While recombinant Tip60 is able to acetylate free but not nucleosomal histones, the Tip60 complex can acetylate either substrate; as observed for Gcn5 and Esa1, participation in a native complex seems to confer chromatin substrate specificity on Tip60. Protein sequencing has now identified a number of subunits in the Tip60 complex, and

some of them have known or potential relationships to transcriptional regulation (Y. Nakatani, unpublished results).

Interestingly, the largest subunit of the Tip60 complex is TRRAP, a transcriptional regulatory protein also found in the human GCN5 and PCAF complexes. Tra1, the yeast homolog of TRRAP, as noted above, is also a component of the yeast HAT complexes SAGA and NuA4, which function in transcriptional activation. It is notable that a TRRAP homolog is present in at least two distinct classes of complexes in both *S. cerevisiae* and humans, and since the PCAF/GCN5 complexes are apparently analogous to SAGA, this leads to the hypothesis that the Tip60 complex may be analogous to NuA4. This possibility is supported by the fact that both catalytic subunits (Esa1 and Tip60) are MYST proteins, but further characterization of NuA4 and the Tip60 complex will be required to determine whether these complexes are otherwise similar, with evolutionarily conserved composition and function. Perhaps relevant to this point, conservation of NuA4 function between *S. cerevisiae* and ciliates has been suggested by a study that identified a *Tetrahymena* nuclear HAT activity that resembles NuA4 in its lysine specificities on nucleosomal histones and ability to promote activated transcription (179).

TFIIIC, HBO1, and other complexes. At least two other complexes have been recovered from human cells and identified as having HAT subunits and activity. One of these, TFIIIC (described earlier), has multiple HAT subunits and the ability to acetylate nucleosomes (109, 133). Purified TFIIIC was able to alleviate chromatin-mediated transcriptional repression in vitro (133), but the direct role of the HAT activities in these assays and in vivo remain to be demonstrated. Another human complex is known to contain MYST protein HBO1 (112) and may interact with ORC, but its other subunits and specific function are still unknown.

In vivo, numerous transcription-related complexes are believed to involve p300/CBP and PCAF (46, 130, 167), bridging interactions between activators and the transcription complex; the same might be said for other coactivator HATs, such as SRC-1, ACTR, and TIF2. However, since some of their interactions may be transient in nature or intimately involve chromatin, many in vivo HAT complexes may be difficult to isolate and characterize in native form in vitro. Finally, the complexes and interacting proteins of several other HATs and putative HATs, such as MOZ and MORF, await identification, as do possible roles of these proteins in transcriptional regulation.

Drosophila MSL Complex

Native HAT complexes from *S. cerevisiae* and human cells have been best characterized, but an interesting complex from another organism—the *Drosophila* MSL complex—has also recently been partially purified and characterized. As mentioned above, the MSL complex is involved in dosage compensation, i.e., increased transcription from the X chromosome in male fruit flies. It contains at least five proteins: MSL1 (male-specific lethal), MSL2, MSL3, MLE (maleless, an RNA-DNA helicase), and MOF, the HAT catalytic subunit. These proteins colocalize to many sites throughout the X chromosome, and mutation of any of the genes encoding them leads to male-specific lethality (150). Studies with coexpressed proteins have defined some of the interactions among the MSL components, and MSL1 was found to have a central role in assembly of the complex (210). In addition, two noncoding RNAs, transcribed from the genes *roX1* and *roX2* (RNA on the X), also colocalize with MSL subunits on the X chromosome (72) and copurify with the MSL complex (166, 217).

Immunoprecipitations from *Drosophila* nuclear extracts have allowed further characterization of the MSL complex in vitro. In HAT assays, the complex acetylated nucleosomes exclusively on lysine-16 of histone H4 in a MOF-dependent manner (217). Since lysine-16-acetylated histone H4 is a hallmark of dosage-compensated chromatin, it is likely that MOF and the MSL complex carry out this acetylation function in vivo as well. It remains to be determined how the MSL complex specifically targets the X chromosome, but one theory is that it gains a foothold through the *roX* genetic loci, which are part of the X chromosome (122). Other aspects of dosage compensation (e.g., how the specific acetylation causes or is associated with increased levels of transcription) also require further analysis, but it is clear that the MSL complex and its HAT subunit are critical to the overall process.

Versions of the SAGA complex (and possibly NuA4) seem to be conserved throughout the eukaryotes; its existence also in *Drosophila* is implied by a recent coimmunoprecipitation of $dTAF_{II}24$ and dGCN5 (78). The MSL complex, however, may be an example of a specialized HAT complex that is not widely conserved. While dosage compensation in *Drosophila* apparently involves HAT-mediated activation, it should be noted that other organisms employ different dosage compensation strategies and thus may not possess analogous HAT complexes. In the nematode *Caenorhabditis elegans*, hermaphrodites experience downregulation of both X chromosomes, and in female mammals, one of the two chromosomes is silenced (165). Instead of an MSL-like complex, these species may therefore use different types of regulatory complexes—which may or may not contain HATs—to control these processes.

FACTOR ACETYLTRANSFERASE (FAT) SUBSTRATES

This review thus far has focused on HATs, but it should be noted that some of these same enzymes are now also known to participate in transcriptional regulation by acetylating proteins other than histones. FAT activities have recently been demonstrated for PCAF, p300/CBP, and $TAF_{II}250$, with transcription-related substrates ranging from activators and coactivators to basal factors and nonhistone chromosomal proteins. These are summarized in Table 2 and described below.

Nonhistone Chromatin Proteins

Histones are by far the best-characterized acetylation substrates within chromatin, but most of the high-mobility-group (HMG) chromatin-associated proteins (reviewed in reference 33) are also known to be acetylated. These proteins participate in transcriptional enhanceosome complexes and higher-order chromatin structure, and recent evidence suggests that HMG acetylation is involved in the regulation of these functions.

HMG1, HMG2, and Sin1. The acetylation of nonhistone protein factors was first described two decades ago, when it was observed that various HMG proteins isolated from vertebrate nuclei had lysine side chains that were postsynthetically acetylated (226). Two of these, HMG1 and HMG2, are related proteins that belong to one of three HMG families. The HMG1/2 proteins are distinguished by their size (about 23 kDa), two DNA-binding motifs (HMG boxes), and an acidic C-terminal region. A second study the following year found that HMG1 was acetylated in vivo at two sites (225). More recently, in vitro enzymatic experiments with partially purified nuclear HAT found that it could acetylate HMG1 and HMG2 in addition to histones (272), and recombinant CBP was able to acetylate rat HMG1 (9). The in vivo importance of these acetylation events and their effects on transcription remain to be studied.

Interestingly, Gcn5 is an apparent candidate for an analo-

TABLE 2. Summary of known FAT substrates[a]

FAT substrate	Known function in vivo	Known FAT enzyme in vitro	Functional effect of acetylation	Observed or predicted effect on transcription
Nonhistone chromatin proteins				
HMG1	Chromatin component	p300/CBP	ND	ND
HMG2	Chromatin component	ND	ND	ND
Yeast Sin1	Transcriptional regulator	Likely Gcn5	ND	ND
HMG14	Nucleosome binding	p300/CBP	Nucleosome binding weakened	ND
HMG17	Nucleosome binding	PCAF	Nucleosome binding weakened	ND
HMG I(Y)	Enhanceosome component	PCAF	Enhanceosome assembly[b]	Positive[b]
		p300/CBP	Enhanceosome disruption	Negative
Transcriptional activators				
p53	Tumor suppressor	PCAF, p300/CBP	Increased DNA binding	Positive
c-Myb	Cell proliferation, differentiation	p300/CBP, GCN5	Increased DNA binding	Positive
GATA-1	Blood cell differentiation	p300/CBP	DNA binding may be affected	Positive
EKLF	Globin gene expression	p300/CBP	Inhibitory domain modified	Positive
MyoD	Muscle differentiation	PCAF	Increased DNA binding	Positive
E2F	Cell cycle control	PCAF	Increased DNA binding	Positive
dTCF	Developmental regulation	p300/CBP	Coactivator interaction disrupted	Negative
HIV Tat	HIV-1 transactivation	PCAF	Increased CDK9 binding	Positive
		p300/CBP	Release from TAR RNA	Positive
Nuclear receptor coactivators	Transcriptional response to hormone signals			
ACTR		p300/CBP	Receptor interaction disrupted	Negative
SRC-1		p300/CBP	ND	ND
TIF2		p300/CBP	ND	ND
General transcription factors	General transcriptional machinery components			
TFIIE		PCAF, p300/CBP, TAF_II250	ND	ND
TFIIF		PCAF, p300/CBP	ND	ND
Importin-α 7, Rch1	Nuclear import	p300/CBP	Increased importin-β binding	ND (may be unrelated to transcription)
α-Tubulin	Microtubule component	ND	ND; stabilized microtubules become acetylated	ND (may be unrelated to transcription)

[a] The FAT substrates characterized in vitro were human, mouse, or rat versions with the exception of yeast Sin1 and *Drosophila* TCF. The other substrates (HMG2 and α-tubulin) have unknown FAT enzymes but were observed to contain one or more acetylated internal lysines in various cell extracts. ND, not determined.
[b] D. Thanos, personal communication.

gous FAT role in *S. cerevisiae*. The yeast protein Sin1 (also known as Spt2), a negative (222) and possibly positive (212) regulator of transcription, has sequence similarity to the HMG1-like proteins of multicellular eukaryotes and is hypothesized to perform a similar function in chromatin (131). When recombinant Sin1 was tested in vitro with partially purified HAT activities from *S. cerevisiae*, Gcn5-dependent acetylation was observed (189). That this occurs in vivo has yet to be demonstrated, but it will be important to explore the functional significance of these observations and their correspondence to HMG1/2 modification.

HMG14 and HMG17. Another early biochemical study demonstrated that the members of a second vertebrate HMG family, HMG14 and HMG17, were acetylated at two and three sites, respectively (224). Only recently has an enzymatic source of one of these acetylation events been determined. When tested in vitro, recombinant HMG17 but not HMG14 acted as an acetylation substrate for PCAF (101). PCAF only acetylated HMG17 at one specific site (lysine-2), as determined by mass spectrometry, and physiological relevance was suggested by the fact that this is the same site predominantly acetylated in vivo (224). HMG14 and HMG17 are known to participate in chromatin by binding specifically to nucleosomes via interaction with the N-terminal tails of their histones (51, 155, 205). Acetylation of HMG17 has now been demonstrated to cause reduced interaction with nucleosomes, suggesting a function for this modification (101). Its transcriptional effect, however, remains to be determined. HMG14 and HMG17 have a positive effect on transcription, as they have been shown to enhance transcription from chromatin templates by unfolding higher-order structure (34), so acetylation may regulate this process.

HMG I(Y). Members of the vertebrate HMG I(Y) family of proteins, comprising HMG I and HMG Y (two isoforms produced from the same gene) and HMG I-C (encoded by a different but closely related gene), have long been known as a type of architectural component of chromosomes (33). In recent years, however, the transcriptional significance of HMG I(Y) has been realized with the finding that it is a component of enhanceosomes (reviewed in reference 39). These are nucleoprotein complexes in which multiple activators and other regulatory proteins interact synergistically at enhancer sequences to bring about activation of specific genes. Perhaps the best characterized of these is formed at the virus-inducible human beta interferon (IFN-β) gene, where two molecules of HMG I(Y) bind enhancer DNA and alter its structure, allowing the recruitment of certain transcription factors (66, 237). This enhanceosome is also known to interact with p300/CBP (167).

Because of the acetylase activities of p300/CBP and its associated PCAF, various IFN-β enhanceosome components were tested in in vitro acetylation assays. Of these factors, only HMG I(Y) was found to be specifically acetylated by both p300/CBP and PCAF (as was HMG I-C, which normally is expressed only in embryonic development but is aberrantly expressed in some cancers) (171). Interestingly, acetylation by p300/CBP, but not PCAF, had negative functional effects in vitro, disrupting the enhanceosome by decreasing HMG I(Y)'s DNA and transcription factor interactions. HMG I(Y) acetylation by p300/CBP was also required for proper IFN-β gene expression in vivo, for both activation and shutoff, while the acetylation function of PCAF was required for full activation but not shutoff.

Transcriptional Activators

Transcriptional activators can be generally defined as proteins that bind to specific sites on promoter DNA and bring about increased transcription of specific genes through interactions with other proteins. As such, they typically contain (i) a DNA-binding domain and (ii) one or more activation domains, which may contact the transcriptional machinery directly or through coactivators and thereby influence transcriptional activity. Acetylation has recently been shown to affect the functions of these domains, either positively or negatively, in a number of activators involved in various cellular and developmental processes.

p53. In higher eukaryotes, p53 is a tumor suppressor that responds to DNA damage by acting as a transcriptional activator of certain cell death-related genes (83, 128, 140). Because of its direct role in such processes as cell cycle arrest and apoptosis, p53's activity as a transcription factor is tightly regulated (79). The various regulatory and functional domains of the 393-residue human p53 protein include N-terminal activation domains, a central DNA-binding domain, and C-terminal tetramerization and regulatory domains. Posttranslational modifications of the C-terminal regulatory regions are one important mode of p53 regulation (163). For example, phosphorylation results in p53 that is competent for DNA binding (111).

Recently, acetylation of p53's C-terminal regulatory domains by both p300/CBP and PCAF has also been demonstrated to be critical for its regulation. p53 interacts directly with p300/CBP (93, 145, 209) and PCAF (148), and in vitro, two lysines (residues 373 and 382) are acetylated specifically by p300/CBP (92), and one (residue 320) is acetylated specifically by PCAF (147, 202). In vitro, these acetylation events were shown to dramatically increase the sequence-specific DNA-binding activity of p53 (92, 148, 202). The acetylated forms of p53 were also observed in vivo, and interestingly, the levels of acetylation increased under DNA-damaging conditions (UV light or ionizing radiation) (147, 202). These results suggest that by acetylation of specific residues, p300/CBP and PCAF positively regulate the activity of p53 as part of the pathway of DNA damage response.

c-Myb. Like p53, the vertebrate protooncogene product c-Myb is an activator whose DNA-binding function, and hence transcriptional activity, is controlled by acetylation. While not a tumor suppressor like p53, c-Myb's natural function is to regulate the proliferation and differentiation of certain types of cells, and improper regulation of or by this protein is associated with oncogenic transformation (238, 263). Recently, human c-Myb was found to act as an acetylation substrate for p300 in vitro and in vivo (239). This acetylation correlated with increased DNA binding of c-Myb in vitro, and in transfection experiments p300 caused elevated c-Myb-dependent transcriptional activation from two promoters in vivo. These results suggest that the transcriptional potential of c-Myb is positively regulated by acetylation of domains affecting DNA binding. Relevant to this, point mutations identified c-Myb's C-terminal negative regulatory domain as containing a subset of the target lysines that may be involved in this regulation. Finally, human GCN5 was also able to acetylate c-Myb in vitro, suggesting that alternate or multiple FAT enzymes may act on c-Myb in the context of the cell (239).

GATA-1. Another acetylation-regulated transcriptional activator important to particular cellular processes of higher eukaryotes is GATA-1, previously known as Eryf1 (65), NF-E1 (253), and GF-1 (156). This transcription factor has a crucial role in the differentiation of certain blood cells, and it binds DNA and interacts with other factors, such as p300/CBP, through a central zinc finger domain. p300 and CBP have recently been shown to acetylate GATA-1 in vitro at two lysine-rich motifs within this central region (24, 110). Furthermore, this acetylation is seen in vivo and serves to increase GATA-1-dependent transcription mediated by p300/CBP, although there are conflicting results as to whether this is caused by augmentation of DNA binding (24, 110).

EKLF. EKLF is another vertebrate blood cell-specific transcription factor recently shown to be acetylated by p300/CBP as part of its regulation. An activator specific to red blood cells (168), EKLF is involved in the developmental switching between embryonic/fetal and adult globin expression (56). In vivo, EKLF can exist in an acetylated form and has been demonstrated to interact with PCAF as well as with p300 and CBP, but in vitro experiments have shown that it acts as an acetylation substrate only for p300/CBP (283). The acetylatable region was mapped to EKLF's inhibitory domain, shown previously to interfere with the function of the nearby DNA-binding domain (44). Furthermore, unlike PCAF, p300/CBP specifically stimulated transcriptional activation by EKLF at a reporter gene in vivo; this effect was apparently acetylation dependent, since the presence of the deacetylase inhibitor trichostatin A significantly increased the activation (283). The precise mechanism for this activation is not yet known, but besides a potential FAT-mediated increase in DNA binding by EKLF, it may also involve histone acetylation, since the presence of EKLF had an impact on chromatin configuration at a globin locus (266).

MyoD. MyoD is another tissue-specific, p300/CBP-associated activator regulated by acetylation, but unlike GATA-1 and EKLF, it is acetylated by PCAF instead of p300/CBP. In muscle cell differentiation (myogenesis), MyoD functions to bind regulatory motifs of promoter DNA and stimulate the transcription of cyclin-dependent kinase inhibitor p21 (94, 95) and muscle-specific genes (259). MyoD was shown to interact with both p300/CBP and PCAF and require them for its activation functions (191, 192), but interestingly, it only required the HAT activity of PCAF, suggesting differential roles for these coactivators (192). Recently, the mode of action of PCAF has been found to involve its direct acetylation of MyoD at three evolutionarily conserved lysine residues (206). This acetylation was observed in vivo and in vitro, and its significance was indicated by the fact that mutation of these residues to nonacetylatable arginines resulted in lack of in vivo transcriptional activation and myogenesis. Proper acetylation, however, appeared to increase MyoD's specific DNA-binding ability in vitro, suggesting a mechanism for the positive effect of PCAF.

E2F. The function of cell cycle-related activator E2F is regulated by acetylation in a manner similar to that observed for MyoD. E2F, typically a heterodimer of an E2F family member and the DP1 protein (138), is known to regulate S-phase-specific genes and thus be required for cell cycle progression (reviewed in reference 1). Interestingly, one of the E2F family members, E2F1, was recently demonstrated to be acetylated by PCAF, and this acetylation has several positive effects on E2F function (159). These modifications were found to occur at three specific lysine residues near the DNA-binding domain of E2F1, and they have the effect of increasing specific DNA binding in vitro and stimulating E2F-mediated transcription in vivo. Furthermore, acetylation seems to stabilize the E2F1 protein and increase its half-life, likely also contributing to the in vivo effectiveness of E2F as an activator (159). However, it should be noted that E2F can also act as a transcriptional repressor when complexed with the Rb protein (260), and a

further experiment demonstrated that this interaction may also negatively regulate the FAT modification of E2F1. Specifically, the HDAC associated with Rb was able to deacetylate E2F1 (159), demonstrating that HDACs and HATs may act antagonistically on their nonhistone substrates, as they do on histones. Finally, the regulatory effect of PCAF may occur with other forms of E2F as well, since the three acetylatable lysine residues are conserved in two other E2F family proteins, E2F2 and E2F3, but this remains to be demonstrated.

dTCF. For the transcription factor substrates described above, acetylation has the net effect of enhancing transcriptional activation. However, an example of a FAT activity participating in transcriptional repression has been demonstrated in the relationship between TCF and CBP in *Drosophila*. As a result of developmental signaling, *Drosophila* TCF (dTCF) normally interacts with its coactivator Armadillo (the ortholog of vertebrate β-catenin), binds DNA at certain enhancer elements, and activates Wnt/Wingless-specific genes (reviewed in references 49 and 61). Interestingly, genetic interactions between CBP and this pathway were recently demonstrated, and moreover, CBP was shown to interact physically with TCF (255). Furthermore, CBP was found to acetylate TCF at lysine-25 within its Armadillo-binding domain, thereby weakening the TCF-Armadillo interaction. The physiological significance of this effect was suggested by a large in vivo increase in TCF-responsive gene expression in a nonacetylatable (Lys-25-Ala) TCF mutant (255). Overall, this study suggests that CBP negatively regulates TCF-mediated transcription by disrupting the activator-coactivator interaction through acetylation. While these experiments were performed with *Drosophila* proteins and embryos, analogous regulation may occur in more complex eukaryotes with regard to the TCF/β-catenin interaction; future studies should reveal whether this particular FAT function is evolutionarily conserved.

HIV Tat. Tat, a transcriptional activator encoded by HIV-1, is another protein whose activation-related functions are affected by acetylation. Unlike conventional activators, this vital protein acts by binding to a region of leader RNA (called TAR) instead of to DNA (18, 54). Tat is known to interact with the human MYST protein Tip60, as mentioned above, but recent studies have shown that it interacts with three other HATs as well: $TAF_{II}250$, p300/CBP, and PCAF (16, 108, 160, 261). Interestingly, the latter two HATs have recently been demonstrated to acetylate Tat at specific, functionally distinct sites (124): PCAF acetylated lysine-28 in the activation domain, whereas p300 acetylated lysine-50 in the TAR RNA-binding domain. In vivo transcription with nonacetylatable alanine substitution mutants and in vitro interaction studies then demonstrated the specific roles of each of these FAT events in Tat-mediated transcription. PCAF-acetylated Tat was found to have an increased affinity (124) for the CDK9/P-TEFb CTD kinase complex (118, 258, 284), suggesting that this acetylation event enhances transcriptional elongation by bringing about hyperphosphorylation of the RNA polymerase II CTD. Acetylation by p300, however, was discovered to decrease the affinity between Tat and the TAR RNA, implicating this modification in the cycled release of Tat from TAR, another important step that apparently allows elongation to proceed (124). Therefore, two acetyltransferases play critical roles in two different steps that promote elongation of Tat-specific transcripts, indicating that HIV-1 has taken advantage of the unique FAT specificities of PCAF and p300 for the regulation of its own gene expression.

Nuclear Receptor Coactivators ACTR, SRC-1, and TIF2

As described earlier, nuclear receptor coactivator ACTR is a HAT that interacts with both p300/CBP and PCAF and activates transcription in response to hormone signals. Remarkably, acetylation of ACTR by p300/CBP has recently been demonstrated to regulate ACTR's activation potential—the first example of one acetylase regulating another by FAT activity (43). By truncation and in vitro acetylation studies, the site of acetylation was identified as the central receptor interaction domain. Amino acid substitution experiments and peptide analysis implicated several lysine residues, all adjacent to an LXXLL motif (100), shown previously to be structurally crucial in the interaction of other proteins with hormone-bound receptors (52). Further experiments showed that acetylation by p300 prevented ACTR from binding receptors, apparently by disturbing key interaction surfaces, ultimately leading to loss of ACTR's activation function (43).

The two other members of the p160 family of nuclear receptor coactivators, SRC-1 and TIF2, can also be efficiently acetylated by p300/CBP in vitro (43). Although the consequences of these acetylation events have not yet been studied, the structural and functional parallels of the three coactivator proteins suggest that they may have a similar mode of regulation. Future investigation should resolve this question.

General Transcription Factors TFIIE and TFIIF

Most of the known targets of FAT activities are activators or coactivators of specific sets of genes, but one study has reported that certain general transcription factors can be acetylated as well. When a number of recombinant human general factors (TFIIA, TFIIB, TBP, TFIIE, and TFIIF) were tested in in vitro assays with PCAF, p300/CBP, and $TAF_{II}250$, it was found that certain components of TFIIE and TFIIF could be acetylated (116). Specifically, the RAP74 and RAP30 subunits of TFIIF were acetylated by PCAF and p300/CBP, and the β subunit of TFIIE was acetylated by these enzymes as well as by $TAF_{II}250$. While these basal factor acetylation events are intriguing, their functions remain to be demonstrated. In vitro transcription studies so far have found no significant effect of TFIIE or TFIIF acetylation on transcription at various promoters (75, 116). Future in vivo studies will likely be required to determine if these factor acetylation events occur physiologically and what, if any, regulatory effects they may have on transcription.

Self-Acetylation and Transcription-Unrelated Substrates

Two other issues that relate to FAT activity but about which relatively little is known are self-acetylation by HATs/FATs and the acetylation of apparently transcription-unrelated substrates, such as α-tubulin and nuclear import factors. Several HATs have been observed to self-acetylate in vitro, including PCAF, p300, Tip60, MORF, Hpa2, and Hpa3. However, it is unknown whether these events have physiological relevance as self-regulation or whether they are merely in vitro artifacts. Further examination of the functional effects of these self-modifications and their occurrence in vivo will be required to resolve this question for each acetylase.

The substrates apparently unrelated to transcription include the microtubule component α-tubulin, known for years to be acetylated in vivo, and two human nuclear import factors, recently demonstrated to be acetylated in vitro by CBP (9). In the case of α-tubulin, lysine-40-acetylated protein has been identified in various organisms (141, 188). This acetylation is known to occur on stabilized microtubules (208), although its

actual functional effect is unclear. Since these acetylated microtubules are cytoplasmic, a direct connection to transcription seems unlikely. Very recently, an in vitro screen for potential acetylation substrates of CBP identified Rch1 and importin-α7 (9), two nuclear import factors that function by binding both to nuclear localization signals on various proteins and to importin-β, which mediates import. For Rch1, acetylation was found to have the effect of enhancing the Rch1/importin-β interaction. This result presents the possibility that this modification could promote nuclear import and is another potential example of acetylation regulating cellular processes other than transcription.

Finally, for all of the FAT substrates described above, it should be noted that acetylation is only one type of modification important for the regulation of some these various factors. For example, p53 is also regulated by phosphorylation, and HMG I(Y) is known to receive various types of modifications. Future studies on the functions of FAT substrates must ultimately address the effects of all relevant modifications in order to provide a complete picture of these proteins' regulation.

CONCLUSIONS AND PERSPECTIVES

As demonstrated by the preceding descriptions of nuclear HATs, their in vivo and in vitro functions, and their transcription-related substrates, acetylation is intimately involved with transcriptional regulation on many levels. For many years, it had been known that there was a correlation between histone-acetylated chromatin and activated transcription; HAT and FAT activities were also recognized and partially purified from various organisms in the last several decades. However, only within the last 5 years have HAT catalytic proteins been identified at a molecular level. The revolutionary finding that a transcriptional adaptor protein, Gcn5, was actually a nuclear HAT—followed quickly by similar discoveries with the well-known coactivator p300/CBP, TFIID subunit $TAF_{II}250$, and other coactivators—established histone acetylation as an apparently ubiquitous mechanism in transcription. This has led to an explosion in HAT-related research, and the lists of HAT proteins, complexes, activities, and substrates continue to grow rapidly.

In many ways, recent advances in molecular biology methods, technology, and informatics have been and will be responsible for the identification and functional characterization of transcriptionally important acetyltransferases. For example, development of the in-gel HAT assay (29) led to the discovery of *Tetrahymena* Gcn5, and the now routine use of protein microsequencing made possible the identification of this and various other HATs. Genome determination and the proliferation of sequence databases are another factor in the discovery of HATs; for example, yeast Esa1 and human PCAF and MORF were originally noticed as database sequences with HAT homology. The imminent completion of human genome sequencing should lead to the identification of additional human HATs. It should be noted that some HATs (e.g., $TAF_{II}250$ and nuclear receptor coactivators) have no recognizable homology to known acetyltransferase motifs and will not be discovered in this way, but the recognition of histone acetylation as a major regulatory mechanism has led to the now widespread use of HAT assays in the characterization of transcription-related proteins, resulting in perhaps unanticipated findings of certain HAT activities (e.g., TFIIIC). This trend is expected to continue in the future.

As the body of information about transcriptional regulation grows and cellular processes previously considered distinct are found to be intricately linked, HAT functional studies are benefiting from multiple, unified scientific approaches. Biochemical and molecular biology techniques are being used to purify HAT complexes and characterize thoroughly their activities and subunits, genetics are providing information about in vivo function, and structural studies, such as the recent GNAT determinations, are giving insights into mechanisms and interactions. These types of investigations will continue, and two recently developed techniques in particular also show promise for future functional determinations of identified HATs and other proteins. Chromatin immunoprecipitation (ChIP) is a way in which chromatin can be retrieved from cells and analyzed for acetylation state or transcriptional proteins at specific genes, providing a wealth of information about in vivo HAT functions and complexes. For a wider functional view, whole-genome analyses with oligonucleotide microarrays have been and will be used to analyze cells' RNA and assess the impact of HAT mutations on the expression of all genes for a given organism or cell type. This has already been performed for Gcn5 and other interesting transcriptional proteins in *S. cerevisiae* (105). In the future, such expression studies will likely be carried out with other HATs in *S. cerevisiae* and also in more complex eukaryotes as the technology and genomic data advance. Thus, in complementary ways, chromatin immunoprecipitation assays and microarrays may provide detailed data about HAT action and other transcriptional regulation under various conditions.

In addition, it should be remembered that histones and regulatory proteins receive a variety of functionally important covalent modifications in vivo, not just acetylation (227). Although this review has focused rather narrowly on chromatin- and transcription-related acetylation, future studies must increasingly address the interplay of multiple modifications with one another, with other activities such as ATP-dependent chromatin remodeling, and with the mechanisms that reverse or antagonize these processes (e.g., deacetylation and chromatin assembly). Recent investigations have established that there are close relationships among all of these functions, but the nature of these must be better defined by future research. Another relevant issue is that the state of chromatin also influences other significant nuclear processes besides transcription, such as DNA replication, recombination, and repair. For example, a recent study revealed that V(D)J recombination of antibody genes is tightly correlated with histone H3 acetylation (162); the connection between such processes and various modes of chromatin alteration will require further investigation. Finally, a largely unexplored frontier in this field is the topic of higher-order chromatin structure, whose effects on transcription must be addressed along with those of nucleosomes in future studies, contributing to the eventual goal of a detailed, overall understanding of the regulation of gene expression in the eukaryotic cell.

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ADDENDUM IN PROOF

The in vitro HAT activity of recombinant full-length MOF has recently been demonstrated by Akhtar and Becker (Mol.

Cell **5:**367–375, 2000). Also, a study by Bergel et al. (J. Biol. Chem. **257:**11514–11520, 2000) has shown that p300 acetylates HMG14 and weakens its interaction with nucleosomes, as observed for PCAF and HMG17. In addition, Marzio et al. (J. Biol. Chem. **275:**10887–10892, 2000) have demonstrated that E2F2 and E2F3, in addition to E2F1, are acetylated by p300/CBP. Finally, Suzuki et al. (Genes Cells **5:**29–41, 2000) have shown that p300 can acetylate Sp1, an activator with which it interacts.

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