Acetylation of general transcription factors by histone acetyltransferases

Axel Imhof*, Xiang-Jiao Yang†, Vasily V. Ogryzko†, Yoshihiro Nakatani†, Alan P. Wolffe* and Hui Ge*

The acetylation of histones increases the accessibility of nucleosomal DNA to transcription factors [1,2], relieving transcriptional repression [3] and correlating with the potential for transcriptional activity *in vivo* [4–7]. The characterization of several novel histone acetyltransferases – including the human GCN5 homolog PCAF (p300/CBP-associated factor) [8], the transcription coactivator p300/CBP [9], and $TAF_{II}250$ [10] – has provided a potential explanation for the relationship between histone acetylation and transcriptional activation. In addition to histones, however, other components of the basal transcription machinery might be acetylated by these enzymes and directly affect transcription. Here, we examine the acetylation of the basal transcriptional machinery for RNA polymerase II by PCAF, p300 and $TAF_{II}250$. We find that all three acetyltransferases can direct the acetylation of TFIIEβ and TFIIF, and we identify a preferred site of acetylation in TFIIEβ. Human TFIIE consists of two subunits, α (p56) and β (p34), which form a heterotetramer ($\alpha_2\beta_2$) in solution ([11], reviewed in [12]). TFIIE enters the preinitiation complex after RNA polymerase II and TFIIF, suggesting that TFIIE may interact directly with RNA polymerase II and/or TFIIF [13,14]. In addition, TFIIE can facilitate promoter melting either in the presence or absence of TFIIH and can stimulate TFIIH-dependent phosphorylation of the carboxy-terminal domain of RNA polymerase II [15–18]. TFIIF has an essential role in both transcription initiation and elongation ([19,20], for review see [21]). We discuss the implications of the acetylation of TFIIEβ and TFIIF for transcriptional control by PCAF, p300 and $TAF_{II}250$.

Addresses: *Laboratory of Molecular Embryology and †Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892-5431, USA.

Correspondence: Hui Ge. E-mail: geh@box-g.nih.gov

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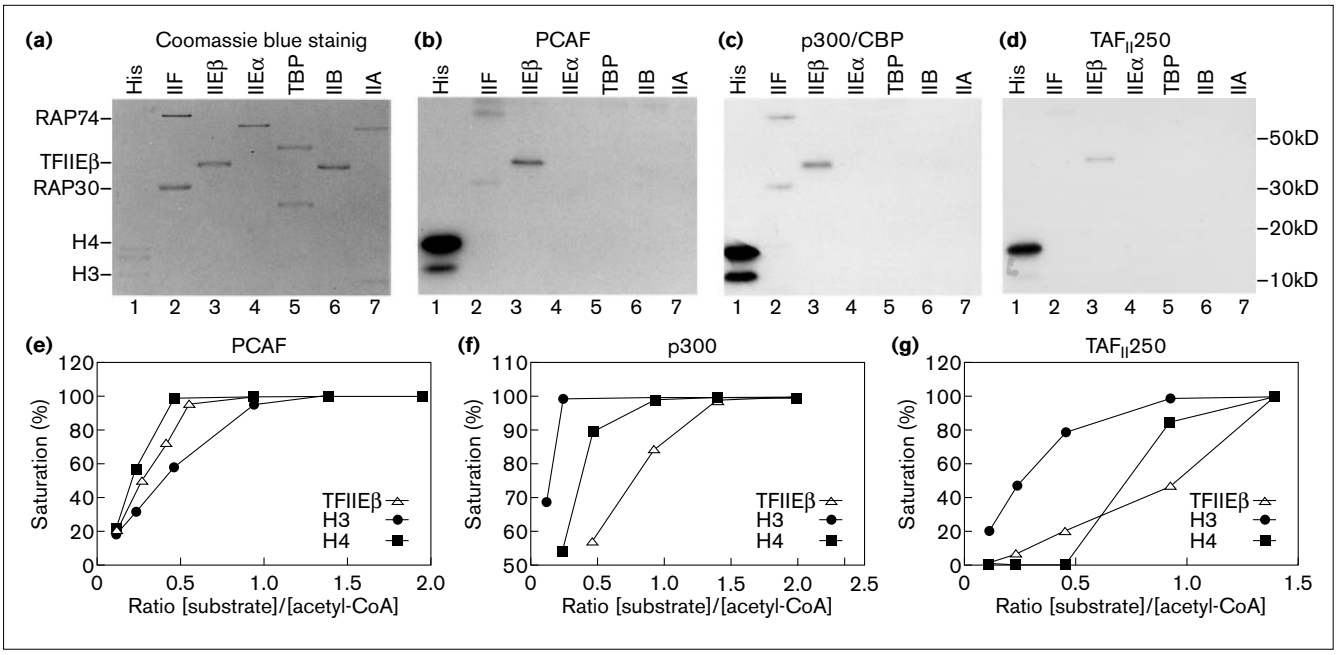
Results and discussion

To ascertain whether any transcription factors can be targeted by histone acetyltransferases, we first normalised the amount of each factor studied both by Coomassie blue staining after SDS-polyacrylamide gel electrophoresis (PAGE; Figure 1a) and by the Bradford method. The recombinant transcription factors studied were TFIIA (p55 and p12 subunits), TFIIB, TATA-binding protein (TBP), the α and β subunits of TFIIE, TFIIF (RAP30 and RAP74 subunits), and the core histones H3 and H4. Each transcription factor was incubated with recombinant PCAF, p300 or $TAF_{II}250$ in the presence of [^{3}H]acetyl CoA (Figure 1b–d). The various acetyltransferases showed substrate selectivity in that histones H3 and H4 were acetylated by all three acetyltransferases, but H4 was modified more efficiently by p300 than by PCAF or $TAF_{II}250$ (Figure 1d). The β subunit of TFIIE was also acetylated by all three enzymes (Figure 1b–d, lane 3). Both subunits of TFIIF, RAP74 and RAP30, were acetylated by PCAF and p300 (Figure 1b,c, lane 2), but $TAF_{II}250$ had little effect on this factor (Figure 1d, lane 2). These observations demonstrate that histone acetyltransferases PCAF and p300 acetylate not only histones H3 and H4 but also the general transcription factors TFIIEβ and TFIIF.

In order to quantify more accurately the efficiency of acetylation of TFIIEβ in comparison to histones H3 and H4, we varied the amounts of substrate proteins while keeping the amount of acetyl CoA constant (Figure 1e–g). Quantification of acetyl CoA incorporation into each substrate under saturating conditions revealed that TFIIEβ is a comparable substrate to histones H3 and H4 for both PCAF and p300 (Table 1). $TAF_{II}250$ shows, however, reduced activity for all tested substrates. The incorporation of acetyl CoA into TFIIEβ catalyzed by PCAF under the saturating conditions ([TFIIEβ] / [acetyl CoA] = 0.5) was 23%, which correlates to 46% of the molecules acetylated if TFIIEβ has one acetylation site (see Figure 2).

To ensure that the acetylation of histones H3 and H4 and TFIIEβ requires enzymatic catalysis and does not result from noncovalent binding, we tested their acetylation by full-length PCAF, by an amino-terminal, non-catalytic PCAF fragment, by the carboxy-terminal PCAF acetyltransferase domain (Figure 2a) and by recombinant yeast HAT1 [22] in a standard acetylation assay. As shown in Figure 2b, wild-type PCAF and the carboxy-terminal acetyltransferase domain fragment acetylated the core histones (lanes 1,3) and TFIIEβ (lanes 6,8), whereas yeast HAT1 acetylated H4 only (lane 4). Under identical conditions, no signal was detected in the reactions containing

Figure 1



Histone acetyltransferases selectively acetylate general transcription factors. Core histones and purified recombinant general transcription factors for RNA polymerase II were normalized by SDS PAGE and visualized by Coomassie blue staining **(a)**, or acetylated in the presence of [^{3}H]acetyl CoA by PCAF **(b)**, by p300/CBP **(c)** or by $TAF_{II}250$ **(d)**. Lane 1: core histones; lane 2: RAP74 and RAP30 subunits of TFIIF; lane 3: TFIIEβ (p34); lane 4: TFIIEα (p56); lane 5: TBP; lane 6: TFIIB; lane 7: TFIIA (p55 and p12). Protein molecular weight standards in kilodaltons (kDa) are indicated on the right. The smaller peptide (~28 kDa) detected in the TBP fraction is an *E. coli* protein copurified from nickel agarose column. Core histones were a gift from A. Gegonne; recombinant general transcription factors and histone acetyltransferases were expressed and purified as described [8–10,23]. Concentrations of each factor were determined by both Bradford method and SDS-PAGE. *In vitro* acetylation assays (20 nl) were carried out with 100 ng of acetyltransferase, 1 ng of substrate, 43 pmol of [^{3}H]acetyl CoA (5.8 Ci/mmol, Amersham Life Science), 10 mM sodium butyrate in the HAT buffer [50 mM Tris-Cl (pH 8.0), 10% glycerol, 1 mM DTT, 1 mM PMSF] at 37°C for 30 min [27]. Reactions were stopped by adding 10 nl of 3× Laemmli buffer and analyzed on an 18% SDS PAA gel. Gels were stained with Coomassie blue, destained, treated with Amplify (Amersham Life Science) for 15 min and exposed for 2–5 days. **(e–g)** Quantification of acetylation. Increasing amounts of TFIIEβ (open triangles), histone H3 (solid circles) and H4 (solid squares) were incubated with a fixed amount of acetyl CoA (43 pmol) in the presence of 100 ng PCAF (e), p300 (f), or $TAF_{II}250$ (g). Corresponding signals are indicated on the left.

the non-catalytic, amino-terminal fragment of PCAF (lanes 2,7) or the control containing no enzyme (lanes 5,10). These results confirm that acetylation of TFIIEβ requires enzymatic function and does not result from non-covalent binding of acetyl CoA to TFIIEβ. It is also apparent that considerable specificity exists in substrate utilization by the different acetyltransferases.

Recently, Kuo *et al.* [23] showed that yGCN5p — a yeast homolog of the human PCAF protein — acetylates histone H3 specifically at lysine 14. A comparison of the sequences of human TFIIEβ and human H3 suggested that the lysine at position 52 in TFIIEβ was a candidate for acetylation by PCAF. This sequence is conserved among human, *Xenopus* and yeast TFIIEβs, implying a functional significance (Figure 2c). We mutated human TFIIEβ Lys 52 to arginine and compared it with wild-type TFIIEβ as a substrate for acetylation. We found that acetylation of the mutant form by PCAF or p300 of TFIIE was severely reduced (more than 90%) compared with wild-type (Figure 2d, compare lanes 1–3 with 4–6, and 7–9 with 10–12).

Our experiments indicate that TFIIE and TFIIF are substrates for acetylation by the acetyltransferases PCAF and p300 (Figures 1,2). Using standard *in vitro* assays, experiments examining the functional role for acetylation of

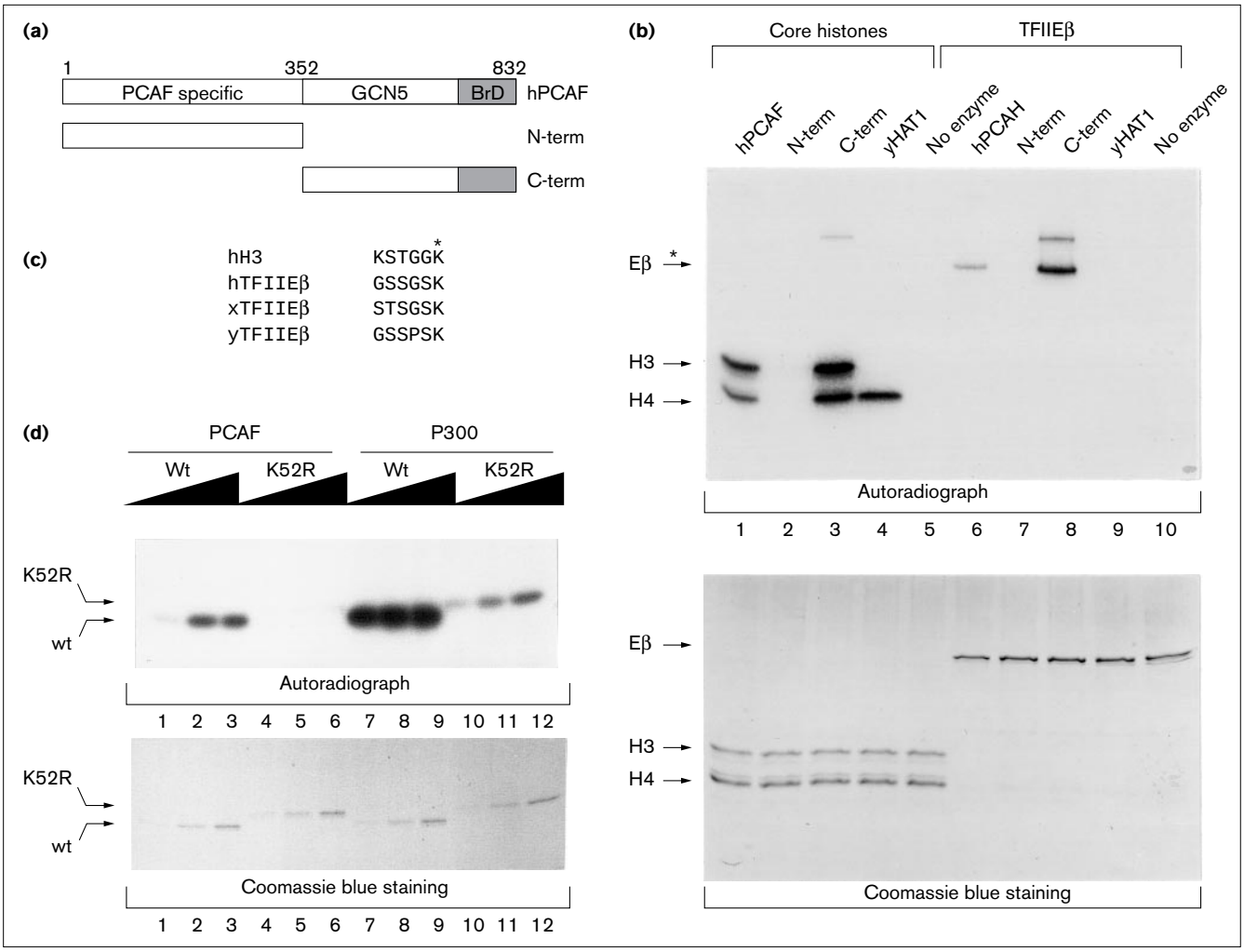
Table 1

Incorporation of acetyl CoA into substrate proteins.

	Substrate incorporation (%)				
Enzyme	RAP74	RAP30	TFIIEβ	H3	H4
PCAF	8 ± 2.8	3 ± 0.8	23 ± 4.5	30 ± 3.0	15 ± 3.2
p300	22 ± 3.7	8 ± 2.8	8 ± 2.8	8 ± 2.8	20 ± 1.5
$TAF_{II}250$	ND	ND	6 ± 3.0	8 ± 1.8	4 ± 0.8

Determination of pmol of acetyl CoA incorporated into the substrate proteins at saturation levels. Percentages of total input acetyl CoA incorporated into the proteins are indicated (errors represent the variation on three independent determinations). ND, not detectable.

Figure 2



The effect of various domains of PCAF on the acetylation of TFIIEβ. **(a)** The amino-terminal and carboxy-terminal domains of PCAF used in these experiments. **(b)** Core histones (lanes 1–5) and TFIIEβ (lanes 6–10) were used as substrates in a standard acetylation assay (see legend to Figure 1). The position of TFIIEβ, H3 and H4 and autoacetylated PCAF (asterisk) is indicated. The various proteins tested for acetyltransferase activity are indicated above the gel lanes. Human PCAF (hPCAF), amino-terminal (N-term) and carboxy-terminal (C-term) fragments of hPCAF and yeast histone acetyltransferase 1 (yHAT1) were used. **(c)** Mutation of the major site of acetylation in TFIIEβ. Alignment of TFIIEβ proteins from human (hTFIIEβ), *Xenopus* (xTFIIEβ) and yeast (yTFIIEβ) and human histone H3 (hH3). The asterisk indicates the conserved lysine 52 in human TFIIEβ that was mutated to an arginine by amplifying the 5′ end of the TFIIEβ cDNA with primers IIE5′ (GCG GAT CCC TGT TGA GAG AAA GGG AG) and K52Rrev (GAT CAG AAT TTT GTC TAG AGC CTG ACG ATCC) and the 3′ end with primers IIE3′ (GCC TCG AGC TAT TTG CTG GAA GTA ATG TC) and K52R (GGA TCG TCA GGC TCT AGA CAA AAT TCT GATC). PCR reactions were then purified and fused together by another amplification step using only primers IIE5′ and IIE3′. The completed PCR reaction was cloned into the *Bam*HI and *Xho*I sites of the bacterial expression vector pRSETA (Invitrogen, San Diego). Recombinant TFIIEβ K52R was expressed in *E. coli* and purified by Ni-column chromatography [24]. **(d)** PCAF and p300 were used in the standard acetylation assay [25] to examine the acetylation of wild-type TFIIEβ (wt) and mutant TFIIEβ (K52R). The mutant migrates slightly differently in SDS gels because of a short amino-terminal extension.

TFIIE and/or TFIIF on various promoters have so far failed to reveal transcriptional stimulation due to acetylation ([24] and data not shown). However, other transcriptional activators or templates such as those assembled into chromatin may be more sensitive to acetylation of the general transcriptional machinery. Our results suggest that PCAF, p300/CBP and $TAF_{II}250$ will have the capacity to modify many other components of the transcriptional regulatory machinery aside from the histones.

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