NF-Y Is Associated with the Histone Acetyltransferases GCN5 and P/CAF*

(Received for publication, October 14, 1997, and in revised form, November 4, 1997)

R. Alexander Currie‡

From the Laboratory of Gene Regulation, The Picower Institute for Medical Research, Manhasset, New York 11030

The ubiquitous transcription factor, NF-Y, plays a pivotal role in the cell cycle regulation of the mammalian cyclin A, cdc25C, and cdc2 genes, in the S-phase activation of the ribonucleotide reductase R2 gene, in addition to its critical role as a key proximal promoter factor in the transcriptional regulation of the albumin, collagen, lipoprotein lipase, major histocompatibility complex class II, and a variety of other eukaryotic and viral genes. In this report, the NF-Y complex has been shown to possess histone acetyltransferase activity through physical association with the related histone acetyltransferase enzymes, human GCN5 and P/CAF in vivo. The assembled NF-YA:B:C complex, and the NF-YB:YC, NF-YB:YC (DNA binding-subunit interaction domain), and NF-YC:YB (DNA binding-subunit interaction domain) heterodimers were sufficient to support stable interaction with human GCN5 in vitro, suggesting that these histone acetyltransferases interact with a unique surface in the ancient YB:YC histone-fold motif. Deletion of either N- or C-terminal regions in human GCN5 disrupted interaction with NF-Y in vitro. In addition, human GCN5 was observed to activate NF-Y in transient transfections in vitro using a natural α2(1) collagen promoter. These results suggest that these associated histone acetyltransferases may serve to modulate NF-Y transactivation potential by aiding disruption of local chromatin structure thereby facilitating NF-Y access to its CCAAT box DNA binding sites.

Chromatin structure plays a vital role in the control and regulation of eukaryotic gene transcription, as nucleosomes are now known to be remodeled during transcription in a dynamic process that involves a number of multicomponent complexes that participate in enzymatic modification of chromatin structures (1). Recent characterization of several ATP-dependent remodeling activities (2–4), and the enzymes that acetylate or deacetylate specific N-terminal histones in the core histones proteins, provide convincing evidence that chromatin structure is significantly involved in transcriptional regulation (5, 6). In addition, a growing subset of known transcriptional cofactors have been shown to possess intrinsic histone acetyltransferase (HAT) activity, as well as established activation domains, and physical links to known DNA binding transcription factors (7–9). The overall importance of HAT activity in transcriptional control mechanisms has recently been underscored by the observation that the DNA binding activity of the tumor suppressor protein, p53, can be regulated through acetylation of specific C-terminal lysine residues by p300/CREB (10).

Nuclear Factor-Y (NF-Y) (11), also known as the CCAAT-binding factor (12) together with its Saccharomyces cerevisiae homolog, HAP2/3/5 (13), is the only known transcription factor whose DNA binding domain is created through the interaction of three heterologous subunits (13–15). Biochemical analyses of the NF-Y complex have demonstrated that the NF-YB:YC subunits associate through a subdomain in the DNA binding-subunit interaction domain (DBD) (16) referred to as the histone-fold “handshake” motif (17, 18), which resembles an α-helical structure first identified in the core histone proteins as primarily responsible for dimerization of the H2A/H2B and H3/H4 histone pairs. The NF-YB:YC histone-fold is most related to histones H2B/H2A, respectively (18), and similarly contains a number of hydrophobic amino acids which project along one face of an α-helix. Both NF-YB:YC and these core histone pairs require strong denaturants to effect their biochemical separation. The NF-YB:YC histone-fold plays a crucial role in creation of a functional NF-Y CCAAT box DNA binding complex as the NF-YA subunit associates only with the YB:YC heterodimer (15).

The yeast protein GCN5 (yGCN5) has long been known to collaborate with yeast GCN4 in the transcriptional regulation of a large number of genes involved in yeast amino acid biosynthesis and to be involved in maximally increasing the transcriptional activity of several respiratory genes which depend on the yeast HAP2/3/4/5 complex (13, 19, 20). yGCN5 is now known to possess intrinsic HAT activity (5) and is thought to acetylate specific N-terminal histone lysines resides as a consequence of its association with additional adaptor proteins in two large multicomponent complexes, referred to as the SAGA complexes (21). In these complexes, additional protein components are thought to modulate yGCN5 substrate specificity, and together these large adaptor structures serve to link upstream activators with the basic RNA polymerase II machinery. Recent cloning and characterization of the human equivalents of these yeast adaptor components (7, 22, 23) has suggested that human GCN5 (hGCN5) is likewise associated with additional protein components and is highly related to another HAT enzyme, P/CAF (7), which has been shown to physically associate with the general transcriptional coactivator, p300/CBP (7), and the hormone receptor cofactor, ACTR (24).

In this report the NF-Y complex has been shown to possess HAT activity in vivo through physical association with the
known HAT enzymes, hGCN5 and P/CAF. hGCN5 activates an NF-Y CCAAT box reporter in *vivo* and associates with the NF-YB:YC histone-fold motif, in *vitro*. This report further identifies the first transcription factor target for hGCN5, suggesting that yGCN5 likewise is associated with the yeast HAP/3/5 complex through the HAP3/5 histone-fold, and thereby suggests a direct functional role for yGCN5 in global yeast respiratory gene regulation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfections**—HeLa and 293 cells were maintained in 10-cm dishes in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (HyClone) and grown at 37 °C, 5% CO2. Full-length human GCN5 (provided by X.-J. Yang and Y. Nakatanai) (7) was cloned into pCDNA3 (Invitrogen) using PCR. pH6 (25) was used to generate a site-directed mutation of the proximal CCAAT box site contained in the 3′(A)1 collagen promoter. Both the wild-type and NF-Y mutant promoters were cloned into the pG3 luciferase vector (Promega) to generate pH6 GL3 and pH6m GL3, respectively, and verified using standard procedures (26). HeLa cells were transfected using the calcium phosphate coprecipitation method and assayed for luciferase and β-galactosidase activities using the Dual-Light assay system (Tropix).

**Recombinant Proteins**—Human GCN5 was cloned into pSETA (Invitrogen) and pGEX2TK (Pharmacia Biotech Inc.) vectors using PCR. The C-terminal deletion mutant of hGCN5 was prepared from GST-hGCN5 by restriction enzyme digestion and contains amino acids 1–332; the "bromodomain" (27) containing N-terminal deletion of amino acids 1–143, respectively (15, 29). The cloning, expression and purification of the full-length GST- and His-NF-YABC subunits has been described previously (30). GST-HMG-Y (20–56) (provided by M. Wegner) (31), GST-hGCN5 and P/CAF were associated with NF-Y in *vivo*. The cloning, expression and purification of full-length GST- and His-NF-YABC subunits has been described previously (30). GST-HMG-Y (20–56) (provided by M. Wegner) (31), GST-D3 (provided by D. Reinberg) (32), and GST-PC4 (provided by R. Roeder) (33) have been described.

**IP-HAT Assays**—HeLa cell nuclear extracts were prepared according to Dignam et al. (34). 293 cells were collected by scraping into 1 ml of ice-cold PBS and precipitated by gentle centrifugation. PBS were replaced with 1 ml of PBS containing 10 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA and the incubation continued at 4 °C for 10 min at 4 °C.

Antibodies were added to 100 μl of either HeLa nuclear extract or 293 whole cell extract at 4 °C for 2 h. Protein A-Sepharose-protein G-Sepharose (15 μl; 1:1) (Pharmacia) was added, and the mixture was rotated overnight at 4 °C. Immune complexes were pelleted by gentle centrifugation and washed six times at 4 °C with 1.5 ml of lysis buffer, followed by two washes with 1 × PBS (1 mg DTT), and two washes with 1 × HAT reaction buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.5% (v/v) Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride). The lysis mixture was incubated on ice for 20 min, then cleared by centrifugation at 12,000 × g for 10 min at 4 °C.

**RESULTS**

During study of accessory protein cofactor interactions with the NF-Y complex (39), a CCAAT box DNA affinity-purified NF-Y fraction was observed to possess histone acetyltransferase activity. To address whether this HAT activity was co-purifying or physically associated with NF-Y in *vivo*, affinity-purified α-NF-YB and α-NF-YA antibodies were used to immunoprecipitate NF-Y derived from HeLa nuclear and 293 whole cell extracts and tested using the IP-HAT assay (8, 35) (Fig. 1, A and B). IP-HAT analysis of the NF-Y complex using either of these subunit directed antibodies resulted in acetylation of the core histone proteins, H3, H2B, H2A, and to a lesser extent, histone H4. Control α-p300 antibodies brought down HAT activity in both cell types in a manner similar to α-NF-Y antibodies, whereas α-E1A antibodies precipitated HAT activity only in 293 extracts and not HeLa extracts as predicted (7, 8, 35). Preincubation of affinity-purified α-YB antibodies with the purified immunogen, GST-YB, effectively blocked specific NF-Y-associated IP-HAT activity (Fig. 1C). Recombinant human GCN5 (hGCN5) acetylated histone H3 predominantly in the liquid HAT assay as has been observed previously with both yeast and human GCN5 (7, 35). Deletion of histone substates or substitution with bovine serum albumin in the IP-HAT assay resulted in no acetylation, whereas both the α-YA and α-YB immunoprecipitates retained specific CCAAT box DNA binding activity, a property of the heterotrimeric NF-Y complex (data not shown).

To identify the protein(s) responsible for the observed NF-Y-associated HAT activity, α-YB immunoprecipitates derived from HeLa extracts were analyzed for the presence of known HAT proteins using Western blot analysis (Fig. 1D). Both hGCN5 and P/CAF HAT proteins were detected in α-YB affinity bead immunoprecipitates using α-hGCN5 affinity-purified antibodies, which were raised against full-length hGCN5 and cross-react with P/CAF (lane 2). P/CAF and NF-YB were also detected in α-YA- and α-YB immunoprecipitates using affinity-purified α-P/CAF antibodies, which were raised against the unique N-terminal region of P/CAF and do not cross-react with hGCN5 (lane 4) and affinity-purified α-YB antibodies (lane 6), respectively. These results suggested that hGCN5 and P/CAF were associated with NF-Y in *vivo* and responsible for the observed HAT activity. P/CAF has been shown to associate with the coactivator, p300/CBP (7), which itself has intrinsic HAT activity, however p300/CBP was not detected in HeLa α-YB immunoprecipitates using Western analysis (data not shown). To examine the possible functional role of hGCN5 in modulating NF-Y transcriptional activity in *vivo*, HeLa cells were transfected with hGCN5 and an NF-Y CCAAT box containing promoter reporter derived from the murine collagen α2(I) gene (25) (Fig. 2). The observed −4–fod activation of this reporter by hGCN5 was dependent on an intact proximal
CCAAT box element, suggesting that hGCN5 functionally interacts and regulates NF-Y transactivation potential on a natural CCAAT box-containing promoter.

To examine the possibility that the NF-Y complex possesses intrinsic HAT activity, individual and specific combinations of recombinant NF-Y subunits were tested for HAT activity using the liquid assay. Neither the complete functional NF-YA:B:C complex, the NF-YB:C heterodimer, nor any individual NF-Y subunit was observed to possess HAT activity (data not shown). An in vitro GST fusion protein "pull-down" assay was developed to determine the specific NF-Y subunit requirements for physical association with hGCN5 (Fig. 3). Recombinant hGCN5 was incubated with the assembled NF-Y complex, the NF-YB:C heterodimer, and individual NF-Y subunits that were tethered to glutathione-agarose beads, then assayed for HAT activity. Using this approach hGCN5 was shown to stably associate with the complete NF-Y complex and the NF-YB:C heterodimer. hGCN5 did not associate with any individual NF-Y subunit, whereas hGCN5 was observed to associate with the full-length YB:YC complex (data not shown) and in heterodimers composed of a full-length subunit and its complementary YB (DBD) or YC (DBD) partner (Fig. 3, lanes 6 and 8, respectively). Recombinant hGCN5 (7, 35), P/CAF (7), and γGCN5 (37) are known to acetylate histone H3 predominantly in the liquid HAT assay when presented with the core histone proteins, whereas acetylation of any of the core histone proteins in nucleosomes by yeast GCN5 requires additional protein components assembled in the large molecular mass SAGA complexes (21, 38). Predominant acetylation of histone H3 by hGCN5 tethered to recombinant NF-Y or NF-YB:YC complexes (Fig. 3) suggests that the histone specificity of hGCN5, and possibly P/CAF, is altered when associated in the native NF-Y complex, since histones H2A and H2B were additionally acetylated by immunoprecipitated NF-Y in the IP-HAT assay (Fig. 1).

In an attempt to map relevant functional domains in hGCN5 that are required for stable interaction with NF-Y, several GST-hGCN5 deletion mutants were tested using an in vitro GST pull-down assay (Fig. 4). NF-YA (DBD) has been used previously to assemble a functional heterotrimeric NF-Y complex and as an individual NF-YA subunit derivative to demonstrate that each stably interacts with a single AT-hook motif present in the non-histone chromosomal proteins, HMG-I(Y) (39) (Fig. 4, A and B, lane 6). Interaction of hGCN5 with NF-Y was dependent on an intact NF-Y complex (Fig. 4A, lane 3), since no specific interaction with YA (DBD) alone was observed.

To examine the possibility that the NF-Y complex possesses intrinsic HAT activity, individual and specific combinations of recombinant NF-Y subunits were tested for HAT activity using the liquid assay. Neither the complete functional NF-YA:B:C complex, the NF-YB:C heterodimer, nor any individual NF-Y subunit was observed to possess HAT activity (data not shown). An in vitro GST fusion protein "pull-down" assay was developed to determine the specific NF-Y subunit requirements for physical association with hGCN5 (Fig. 3). Recombinant hGCN5 was incubated with the assembled NF-Y complex, the NF-YB:C heterodimer, and individual NF-Y subunits that were tethered to glutathione-agarose beads, then assayed for HAT activity. Using this approach hGCN5 was shown to stably associate with the complete NF-Y complex and the NF-YB:C heterodimer. hGCN5 did not associate with any individual NF-Y subunit, whereas hGCN5 was observed to associate with the full-length YB:YC complex (data not shown) and in heterodimers composed of a full-length subunit and its complementary YB (DBD) or YC (DBD) partner (Fig. 3, lanes 6 and 8, respectively). Recombinant hGCN5 (7, 35), P/CAF (7), and γGCN5 (37) are known to acetylate histone H3 predominantly in the liquid HAT assay when presented with the core histone proteins, whereas acetylation of any of the core histone proteins in nucleosomes by yeast GCN5 requires additional protein components assembled in the large molecular mass SAGA complexes (21, 38). Predominant acetylation of histone H3 by hGCN5 tethered to recombinant NF-Y or NF-YB:YC complexes (Fig. 3) suggests that the histone specificity of hGCN5, and possibly P/CAF, is altered when associated in the native NF-Y complex, since histones H2A and H2B were additionally acetylated by immunoprecipitated NF-Y in the IP-HAT assay (Fig. 1).

In an attempt to map relevant functional domains in hGCN5 that are required for stable interaction with NF-Y, several GST-hGCN5 deletion mutants were tested using an in vitro GST pull-down assay (Fig. 4). NF-YA (DBD) has been used previously to assemble a functional heterotrimeric NF-Y complex and as an individual NF-YA subunit derivative to demonstrate that each stably interacts with a single AT-hook motif present in the non-histone chromosomal proteins, HMG-I(Y) (39) (Fig. 4, A and B, lane 6). Interaction of hGCN5 with NF-Y was dependent on an intact NF-Y complex (Fig. 4A, lane 3), since no specific interaction with YA (DBD) alone was observed.

To examine the possibility that the NF-Y complex possesses intrinsic HAT activity, individual and specific combinations of recombinant NF-Y subunits were tested for HAT activity using the liquid assay. Neither the complete functional NF-YA:B:C complex, the NF-YB:C heterodimer, nor any individual NF-Y subunit was observed to possess HAT activity (data not shown). An in vitro GST fusion protein "pull-down" assay was developed to determine the specific NF-Y subunit requirements for physical association with hGCN5 (Fig. 3). Recombinant hGCN5 was incubated with the assembled NF-Y complex, the NF-YB:C heterodimer, and individual NF-Y subunits that were tethered to glutathione-agarose beads, then assayed for HAT activity. Using this approach hGCN5 was shown to stably associate with the complete NF-Y complex and the NF-YB:C heterodimer. hGCN5 did not associate with any individual NF-Y subunit, whereas hGCN5 was observed to associate with the full-length YB:YC complex (data not shown) and in heterodimers composed of a full-length subunit and its complementary YB (DBD) or YC (DBD) partner (Fig. 3, lanes 6 and 8, respectively). Recombinant hGCN5 (7, 35), P/CAF (7), and γGCN5 (37) are known to acetylate histone H3 predominantly in the liquid HAT assay when presented with the core histone proteins, whereas acetylation of any of the core histone proteins in nucleosomes by yeast GCN5 requires additional protein components assembled in the large molecular mass SAGA complexes (21, 38). Predominant acetylation of histone H3 by hGCN5 tethered to recombinant NF-Y or NF-YB:YC complexes (Fig. 3) suggests that the histone specificity of hGCN5, and possibly P/CAF, is altered when associated in the native NF-Y complex, since histones H2A and H2B were additionally acetylated by immunoprecipitated NF-Y in the IP-HAT assay (Fig. 1).

In an attempt to map relevant functional domains in hGCN5 that are required for stable interaction with NF-Y, several GST-hGCN5 deletion mutants were tested using an in vitro GST pull-down assay (Fig. 4). NF-YA (DBD) has been used previously to assemble a functional heterotrimeric NF-Y complex and as an individual NF-YA subunit derivative to demonstrate that each stably interacts with a single AT-hook motif present in the non-histone chromosomal proteins, HMG-I(Y) (39) (Fig. 4, A and B, lane 6). Interaction of hGCN5 with NF-Y was dependent on an intact NF-Y complex (Fig. 4A, lane 3), since no specific interaction with YA (DBD) alone was observed.
GCN5 and P/CAF Interact with NF-Y in Vivo

Fig. 3. NF-Y and hGCN5 associate through the highly conserved DBD elements of the NF-YB:YC heterodimer. The subunit requirements for stable interaction of NF-Y and hGCN5 were determined using an in vitro GST fusion protein interaction assay. hGCN5 was incubated with the indicated individual or combinations of NF-Y subunits bound to glutathione-agarose beads, or controls, then assayed for HAT activity. Lane 1, glutathione-agarose beads; lane 2, GST; lane 3, GST-YA (full length); lane 4, GST-YA (His-B-C); full-length complex; lane 5, GST-YB (full length); lane 6, GST-YB:YC (DBD); lane 7, GST-YC (full length); lane 8, GST- 19 YC:YB (DBD). The control hGCN5 HAT reaction is shown at the left, and the position of histone H3 is denoted at the right.

DISCUSSION

Both single nucleosomes and higher order chromatin structures are generally thought to represent structural impediments to gene transcription (1). Recent isolation of chromatin remodeling activities has provided new insights into the mechanisms responsible for altering chromatin structure during transcription and the means to begin approaching questions regarding the targeting of specific activities to specific promoters (2–6). Further refinement in the crystal structure of the core nucleosome particle (40) has now supported earlier results (41), which together suggest the N-terminal histone tails are involved in nucleosome-nucleosome interactions and are involved in maintaining higher order chromatin structure. Together these observations now suggest that acetylation of the N-terminal histone lysines resides is involved in disrupting the structure between nucleosomes in local regions in chromatin thereby facilitating access of transcription factors to specific promoters. In addition, recent reports have demonstrated that p53 is a substrate for p300 (10), and the HAT enzymes, p300, P/CAF, and TAF1250, are capable of acetylating components of the general RNA polymerase II machinery (i.e. TFIIIE and TFIIIF) (42). These results suggest that HAT enzymes play additional roles in modulating the DNA binding activity of an important tumor suppressor protein, and the functional activity of general initiation factors, and further suggest important implications for their role as cofactors in modulating upstream transcription factor transactivation potential.

This report identifies the NF-Y complex as the first mammalian transcription factor target for the human acetyltransferase, GCN5, the first DNA-binding transcription factor associated with P/CAF, in vivo, and maps the site of interaction of hGCN5 to the highly conserved DBD elements of the NF-YB:C heterodimer. Anti-YB immunoprecipitates, which contain the NF-Y complex, were observed to be associated with the two highly related HAT enzymes, GCN5 and P/CAF. The C-terminal region of P/CAF is 86% identical on the amino acid level to hGCN5 (7), which includes both the region responsible for HAT activity and the bromodomain, a motif thought to be involved in additional protein-protein interactions (5, 27). The N-terminal region of P/CAF is unique, and α-P/CAF antibodies (7) that recognize both N- and C-terminal regions were observed to detect P/CAF in α-YB immunoprecipitates (Fig. 1D). P/CAF has been shown to be associated with two cofactors, p300/CREB (7) and ACTR (24); however, the site(s) of physical interaction in either case has not been identified. While P/CAF is known to interact with p300/CREB, hGCN5 does not (7), and presently no mammalian transcription factor has been shown to interact with both hGCN5 and P/CAF. NF-Y is the first DNA-binding transcription factor shown to be associated with P/CAF and hGCN5. In vitro binding studies between NF-Y and hGCN5 strongly suggest that P/CAF interacts with NF-Y likewise through its C-terminal region, which is highly related to hGCN5. Presently it is not known if these NF-Y: HAT complexes exhibit differential activities with regard to specific NF-Y-responsive promoters in vivo, and if the composition of NF-Y: HAT complexes differ with regard to additional protein components.

Comparison of immunoprecipitated NF-Y-associated HAT activity with in vitro reconstituted NF-Y:hGCN5 HAT activity showed an altered histone substrate specificity. Both histone H2A and H2B were observed to be acetylated when presented to NF-Y immunoprecipitates in addition to the predominant product, H3, while in vitro reconstituted complexes acetylated histone H3 exclusively. These results suggest that native NF-Y may be associated with additional protein components in vivo, in a manner possibly analogous to the yeast SAGA complexes that contain yGCN5, in addition to the characterized components, Spt 3/7/20, ADA2, ADA3, and other unknown components (21). hGCN5, yGCN5, and P/CAF are known to acetylate histone H3 predominantly when presented with the core histone proteins and to be unable to acetylate histones in isolated nucleosomes in the absence of additional protein components.

Fig. 4. Deletion of either N- or C-terminal regions of hGCN5 disrupts interaction with NF-Y. An in vitro glutathione-agarose bead pull-down assay was used to test several GST-hGCN5 deletion mutants for their ability to stably interact with NF-Y. A. 32p-P-YA (DBD) assembled in the NF-Y complex was used to measure interaction with GST-hGCN5 and several derivatives. B, 32p-P-YA (DBD) was tested alone as probe. GST-HMG-Y (20–56) contains two AT-hook motifs (31), and GST-hGCN5 and several derivatives. Assembled in the NF-Y complex was used to measure interaction with NF-Y responsive promoters (40) has now supported earlier results (41), which together suggest the N-terminal histone tails are involved in nucleosome-nucleosome interactions and are involved in maintaining higher order chromatin structure. Together these observations now suggest that acetylation of the N-terminal histone lysines residues is involved in disrupting the structure between nucleosomes in local regions in chromatin thereby facilitating access of transcription factors to specific promoters. In addition, recent reports have demonstrated that p53 is a substrate for p300 (10), and the HAT enzymes, p300, P/CAF, and TAF1250, are capable of acetylating components of the general RNA polymerase II machinery (i.e. TFIIIE and TFIIIF) (42). These results suggest that HAT enzymes play additional roles in modulating the DNA binding activity of an important tumor suppressor protein, and the functional activity of general initiation factors, and further suggest important implications for their role as cofactors in modulating upstream transcription factor transactivation potential.

This report identifies the NF-Y complex as the first mammalian transcription factor target for the human acetyltransferase, GCN5, the first DNA-binding transcription factor associated with P/CAF, in vivo, and maps the site of interaction of hGCN5 to the highly conserved DBD elements of the NF-YB:C heterodimer. Anti-YB immunoprecipitates, which contain the NF-Y complex, were observed to be associated with the two highly related HAT enzymes, GCN5 and P/CAF. The C-terminal region of P/CAF is 86% identical on the amino acid level to hGCN5 (7), which includes both the region responsible for HAT activity and the bromodomain, a motif thought to be involved in additional protein-protein interactions (5, 27). The N-terminal region of P/CAF is unique, and α-P/CAF antibodies (7) that recognize both N- and C-terminal regions were observed to detect P/CAF in α-YB immunoprecipitates (Fig. 1D). P/CAF has been shown to be associated with two cofactors, p300/CREB (7) and ACTR (24); however, the site(s) of physical interaction in either case has not been identified. While P/CAF is known to interact with p300/CREB, hGCN5 does not (7), and presently no mammalian transcription factor has been shown to interact with both hGCN5 and P/CAF. NF-Y is the first DNA-binding transcription factor shown to be associated with P/CAF and hGCN5. In vitro binding studies between NF-Y and hGCN5 strongly suggest that P/CAF interacts with NF-Y likewise through its C-terminal region, which is highly related to hGCN5. Presently it is not known if these NF-Y: HAT complexes exhibit differential activities with regard to specific NF-Y-responsive promoters in vivo, and if the composition of NF-Y: HAT complexes differ with regard to additional protein components.

Comparison of immunoprecipitated NF-Y-associated HAT activity with in vitro reconstituted NF-Y:hGCN5 HAT activity showed an altered histone substrate specificity. Both histone H2A and H2B were observed to be acetylated when presented to NF-Y immunoprecipitates in addition to the predominant product, H3, while in vitro reconstituted complexes acetylated histone H3 exclusively. These results suggest that native NF-Y may be associated with additional protein components in vivo, in a manner possibly analogous to the yeast SAGA complexes that contain yGCN5, in addition to the characterized components, Spt 3/7/20, ADA2, ADA3, and other unknown components (21). hGCN5, yGCN5, and P/CAF are known to acetylate histone H3 predominantly when presented with the core histone proteins and to be unable to acetylate histones in isolated nucleosomes in the absence of additional protein components.
GCN5 and P/CAF Interact with NF-Y in Vivo

(7, 37). Recent isolation of hADA2, the human homolog to the yeast adaptor protein, yADA2 (22), suggests that some fraction of both hGCN5 and P/CAF may be associated in vivo with hADA2 and mammalian equivalents of the SAGA complexes. Further analysis of α-NF-Y immunoprecipitates will determine if hADA2 is present and the identity of additional protein components that may play a role in regulating both hGCN5 and P/CAF histone substrate specificity.

Mutational analysis of the NF-YB subunit has established that the YC interaction domain and the region required for DNA binding activity largely overlap in the ~90-amino acid YB (DBD) (16). In contrast, the NF-YA (DBD) element is more clearly defined and contains separable subdomains used for DNA binding and interaction with the YB:YC heterodimer. The YB:YC histone-fold motifs appear to play crucial roles in subunit interactions through creation of unique surfaces for interaction with the YA (DBD) and with the HAT enzymes, hGCN5 and P/CAF. The YB:YC histone-fold may make additional non-specific contacts with DNA sequences flanking the CCAAT box, in a manner analogous to the H2B:H2A histone pair, whereas the YA (DBD) may make the majority of sequence-specific DNA contact in the CCAAT box, in addition to providing binding surfaces for HMG-1(Y) and PC4/p15 (39). Clearly, x-ray crystallographic analyses of the NF-Y (DBD) regions in association with CCAAT box DNA and these newly identified HAT enzymes in the future will further our understanding of how these proteins are structurally organized and provide additional insights into how they function in vivo.

Acknowledgments—I am grateful to X.-J. Yang and Y. Nakatani for kindly providing their hGCN5 and P/CAF reagents and to Drs. D. McNabb, L. Guarante, X.-Y. Yang, and Y. Nakatani for their comments and suggestions.

REFERENCES
1. Grunstein, M. (1997) Nature 389, 349–352
2. Peterson, C. L., and Tamkun, J. W. (1995) Trends Biochem. Sci. 20, 143–146
3. Tsukiyama, T., and Wu, C. (1995) Cell 83, 1011–1020
4. Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lecomte, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R. D. (1996) Cell 87, 1249–1260
5. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) Cell 84, 843–851
6. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) Science 272, 408–411
7. Yang, X.-J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324
8. Bannister, A. J., and Kouzardes, T. (1996) Nature 384, 641–643
9. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. M., and Nakatani, Y. (1990) Cell 87, 853–859
10. Gu, W., and Roeder, R. G. (1997) Cell 87, 595–606
11. Dorn, A., Bollekeens, J., Staub, A., Benoist, C., and Mathis, D. (1987) Cell 50, 863–872
12. Maity, S. N., Golombok, P. T., Karsenty, G., and de Crombrugghe, B. (1988) Science 241, 582–585
13. McNabb, D. S., Xing, Y., and Guarente, L. (1995) Genes Dev. 9, 47–58
14. Maity, S. N., Sinha, S., Rutherhouser, E. C., and de Crombrugghe, B. (1992) J. Biol. Chem. 267, 16574–16580
15. Sinha, S., Maity, S. N., Luo, J., and de Crombrugghe, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1624–1628
16. Sinha, S., Kim, I.-S., Sohn, K.-Y., de Crombrugghe, B., and Maity, S. N. (1996) Mol. Cell. Biol. 16, 328–337
17. Arents, G., Buringame, R. W., Wang, B.-C., Love, W. E., and Moudrianakis, E. N. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10148–10152
18. Banerjee, A. D., Arents, G., Moudrianakis, E. N., and Lundeheim, D. (1995) Nucleic Acids Res. 23, 2685–2691
19. Georgakopoulos, T., and Thireos, G. (1992) EMBO J. 11, 4145–4152
20. Georgakopoulos, T., Gounalaki, N., and Thireos, G. (1995) Mol. Gen. Genet. 246, 723–728
21. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohka, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) Genes Dev. 11, 1640–1650
22. Candau, R., Moore, P. A., Wang, L., Barlev, N., Ying, C. Y., Rosen, C. A., and Berger, S. L. (1996) Mol. Cell. Biol. 16, 593–602
23. Wang, L., Mizzen, C., Ying, C., Candau, R., Barlev, N., Brownell, J., Allis, C. D., and Berger, S. L. (1997) Mol. Cell. Biol. 17, 519–527
24. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalov, L. E., Nakatani, Y., and Evans, R. M. (1997) Cell 90, 569–580
25. Coursy, T., Maity, S. N., and de Crombrugghe, B. (1995) J. Biol. Chem. 270, 468–475
26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Haynes, S. R., Sollard, C., Winston, F., Beck, S., Trowsdale, J., and Dawid, I. B. (1992) Nucleic Acids Res. 20, 2683–2693
28. Smith, D. B., and Johnson, K. S. (1988) Gene (Amst.) 70, 31–40
29. van Huijsduijnen, R. H., Li, X.-Y., Black, D., Matthes, H., Benoist, C., and Mathis, D. (1990) EMBO J. 9, 3119–3127
30. Nakshatri, H., Bhat-Nakshatri, P., and Currie, R. A. (1996) J. Biol. Chem. 271, 28784–28791
31. Leger, H., Schek, E., Renner, K., Grummt, F., and Wegner, M. (1995) Mol. Cell. Biol. 15, 3738–3747
32. Inostroza, J. A., Mermelstein, F. H., Ha, I., Lane, W. S., and Reinberg, D. (1992) Cell 70, 477–489
33. Ge, H., and Roeder, R. G. (1994) Cell 78, 513–523
34. Dignam, J. D., Lebowitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1488
35. Mizzen, C. A., Yang, X.-J., Kobubo, T., Brownell, J., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzardes, T., Nakatani, Y., and Allis, C. D. (1996) Cell 87, 555–565
36. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 311–452, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
37. Kuo, M.-H., Brownell, J. E., Sobel, R. E., Ranalli, T. A., Cook, R. G., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) Nature 383, 269–272
38. Candau, R., Zhou, J., Allis, C. D., and Berger, S. L. (1997) EMBO J. 16, 555–566
39. Currie, R. A. (1997) J. Biol. Chem. 272, 30880–30888
40. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Nature 389, 251–260
41. van Holde, K., and Zlatanova, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10548–10555
42. Imhof, A., Yang, X.-J., Ogryzko, V. V., Nakatani, Y., Wolfe, A. P., and Ge, H. (1997) Curr. Biol. 7, 689–692