Molecular Cell, Vol. 9, 575–586, March, 2002, Copyright ©2002 by Cell Press

Structural Basis of Lysine-Acetylated HIV-1 Tat Recognition by PCAF Bromodomain

Shiraz Mujtaba,[1] Yan He,[1] Lei Zeng,[1] Amjad Farooq,[1] Justin E. Carlson,[1] Melanie Ott,[2] Eric Verdin,[3] and Ming-Ming Zhou[1,4]
[1]Structural Biology Program
Department of Physiology and Biophysics
Mount Sinai School of Medicine
New York University
New York, New York 10029
[2]Applied Tumor Virology
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld, 242
Heidelberg, D-69120
Germany
[3]Gladstone Institute of Virology and Immunology
University of California, San Francisco
365 Vermont Street
San Francisco, California 94103

Summary

The human immunodeficiency virus type 1 (HIV-1) *trans*-activator protein Tat stimulates transcription of the integrated HIV-1 genome and promotes viral replication in infected cells. Tat transactivation activity is dependent on lysine acetylation and its association with nuclear histone acetyltransferases p300/CBP (CREB binding protein) and p300/CBP-associated factor (PCAF). Here, we show that the bromodomain of PCAF binds specifically to HIV-1 Tat acetylated at lysine 50 and that this interaction competes effectively against HIV-1 TAR RNA binding to the lysine-acetylated Tat. The three-dimensional solution structure of the PCAF bromodomain in complex with a lysine 50-acetylated Tat peptide together with biochemical analyses provides the structural basis for the specificity of this molecular recognition and reveals insights into the differences in ligand selectivity of bromodomains.

Introduction

The human immunodeficiency virus type 1 (HIV-1) protein Tat is an atypical *trans*-activator of transcription which functions through binding to an RNA element known as the transactivation responsive region (TAR), located in the retroviral long-terminal repeat (LTR) (Cullen, 1998; Jeang et al., 1999; Karn, 1999). Tat binds to TAR RNA with high affinity but transiently (Keen et al., 1997; Rana and Jeang, 1999). Dissociation of Tat from TAR RNA facilitates Tat association with the assembled RNA polymerase II (RNAPII) complex (Deng et al., 2000; Kiernan et al., 1999). The latter process enables the transcriptional machinery complex to elongate efficiently on the viral DNA template in order to produce full-length HIV transcripts during viral productive replication in infected cells (Adams et al., 1994; Garber and Jones, 1999).

While the detailed molecular mechanisms underlying Tat dissociation from TAR RNA and its transactivation of transcription of the integrated HIV-1 genome remain elusive, increasing lines of evidence suggest that Tat activity requires its association with several multiprotein complexes, which include the cyclinT1/cyclin-dependent kinase 9 (CDK9) complex (Jones, 1997; Wei et al., 1998) and histone acetyltransferase (HAT) transcriptional coactivators, p300/CBP (CREB binding protein), and p300/CBP-associated factor (PCAF) (Benkirane et al., 1998; Deng et al., 2000; Hottiger and Nabel, 1998). Recruitment of CDK9 through the N-terminal cysteine-rich region of Tat results in hyperphosphorylation of the C-terminal domain of RNAPII that increases elongation efficiency of HIV-1 transcription (Wei et al., 1998). Recently, it has been shown that Tat activity is dependent on acetylation by p300/CBP at K50 located in the C-terminal arginine-rich motif (ARM) (Kiernan et al., 1999; Ott et al., 1999), a region that is also important for TAR RNA binding and nuclear localization. Mutation of K50 to arginine, a conserved amino acid substitution that retains the positive charge but prevents acetylation by p300, markedly decreases the synergistic activation of the HIV-1 promoter by Tat and p300 (Kiernan et al., 1999; Ott et al., 1999). Tat acetylation at K50 results in its dissociation from TAR RNA and promotes formation of a multiprotein complex comprised of Tat, p300/CBP, and PCAF (Benkirane et al., 1998; Deng et al., 2000). Furthermore, it has been shown that the HAT activity of PCAF is preferentially required for Tat transactivation of transcription of the integrated but not the unintegrated HIV-1 LTRs (Benkirane et al., 1998).

Protein lysine acetylation is emerging as a central mechanism in regulation of chromatin remodeling and transcriptional activation (Jenuwein and Allis, 2001; Kouzarides, 2000; Strahl and Allis, 2000). Bromodomains, an extensive family of conserved protein modules found in many chromatin-associated proteins and in nearly all nuclear histone acetyltransferases (Brownell and Allis, 1996; Haynes et al., 1992; Jeanmougin et al., 1997; Tamkun et al., 1992), have been recently discovered to function as acetyl-lysine binding domains (Dhalluin et al., 1999; Hudson et al., 2000; Jacobson et al., 2000; Owen et al., 2000). This new finding suggests a novel mechanism for regulating protein-protein interactions via lysine acetylation (Dyson et al., 2001; Jenuwein and Allis, 2001; Strahl and Allis, 2000; Winston and Allis, 1999). This new mechanism supports the hypothesis that bromodomains could contribute to highly specific histone acetylation by tethering transcriptional HATs to specific chromosomal sites (Brownell and Allis, 1996; Manning et al., 2001; Travers, 1999), and to the assembly and activity of multiprotein complexes of chromatin remodeling such as SAGA and NuA4 (Brown et al., 2001; Sterner et al., 1999). However, because no specific, biologically relevant binding sites had been reported for any particular bromodomain, the major question of ligand specificity of bromodomains still remains unanswered.

[4]Correspondence: zhoum@inka.mssm.edu

In efforts to determine the mechanisms of action of Tat in transactivation of HIV-1 transcription, we investigated whether the interaction of the activated, lysine-acetylated Tat with the nuclear HAT transcriptional cofactors p300/CBP and PCAF involves any of the bromodomains of the latter proteins. Here, we report that the bromodomain of PCAF but not CBP can specifically recognize the lysine-acetylated Tat at K50 (not K51 or K28), and this interaction competes effectively against TAR RNA binding to the acetylated Tat. We have also determined the three-dimensional structure of the PCAF bromodomain in complex with a lysine-acetylated peptide derived from Tat at K50 by using nuclear magnetic resonance (NMR) spectroscopy. NMR structural and biochemical analyses were further used to gain structural insights into this important molecular recognition as well as the differences in ligand selectivity of different bromodomains.

Results and Discussion

PCAF Bromodomain Recognition of Lysine-Acetylated HIV-1 Tat at K50

To test whether Tat-p300/CBP-PCAF association involves bromodomains of the histone acetyltransferase transcriptional coactivators, we performed an in vitro binding assay by using recombinant and purified GST-fusion bromodomains and lysine-acetylated peptides derived from known acetylation sites in Tat at K28 and K50. A lysine-acetylated Tat peptide containing AcK50 (SYGR-AcK-KRRQR, where AcK is an N^{ϵ}-acetyl-lysine) showed no detectable interactions with either bromodomains or bromodomain and PHD finger (Aasland et al., 1995) tandem modules from CBP or TIF1β (transcriptional intermediary factor 1β, also named KAP-1 and KRIP-1) (Friedman et al., 1996) (Figure 1A). Strikingly, the same Tat peptide bound tightly to the bromodomain of PCAF, which shares high sequence homology to CBP bromodomain (Jeanmougin et al., 1997). The binding is dependent on acetylation of K50 on Tat. Neither of these bromodomains interacted with an acetylated Tat peptide derived from K28 (TNCYCK-AcK-CCFH) (data not shown), highlighting the selective nature of PCAF bromodomain recognition of the Tat AcK50 sequence.

We performed an NMR study in order to determine the specificity of PCAF bromodomain binding to lysine-acetylated Tat. As anticipated, PCAF bromodomain did not bind to Tat AcK28 peptide, nor did CBP bromodomain to either Tat AcK28 or AcK50 peptide. In contrast, PCAF bromodomain bound to Tat AcK50 peptide with high affinity and caused extensive chemical shift perturbations of protein amide resonances, significantly greater than those seen with an acetylated peptide derived from histone H4 at K16, as shown in 2D ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectra (Figure 1B). This observation agrees with the differences in dissociation constants (K_D), determined by NMR titration to be $\sim$10 μM and $>$300 μM for the former and latter complexes, respectively. These results argue that PCAF bromodomain binding to H4 peptide is largely limited to the acetyl-lysine, whereas its recognition of Tat most likely involves additional interactions with residues flanking AcK50.

To assess the biological relevance of the PCAF bromodomain and Tat interaction to the activation of Tat transcriptional activity by PCAF and p300/CBP (Benkirane et al., 1998; Kiernan et al., 1999), we performed cell transfection experiments and measured their combined effect on the activity of the HIV-1 promoter using an HIV-1 LTR-luciferase reporter gene assay (Bieniasz et al., 1998; Madore and Cullen, 1993). As shown in Figure 1C, synergistic activation of Tat-mediated transcription of the HIV-1 promoter in human 293T cells is dependent upon both PCAF and CBP. The latter HAT transcriptional coactivator has been recently shown to be responsible for lysine acetylation of Tat at K50 that is required for Tat activation (Kiernan et al., 1999; Ott et al., 1999). More importantly, our data show that cotransfection of the PCAF bromodomain but not the CBP bromodomain resulted in a significant reduction of the synergistic activation of Tat by PCAF and CBP, likely due to an effective competition of the PCAF bromodomain against the full-length PCAF binding to Tat. Collectively, our in vivo transfection study further confirms the highly specific nature of PCAF bromodomain/Tat recognition and highlights the functional importance of this bromodomain interaction in the synergistic PCAF- and CBP-induced activation of Tat transcriptional activity in HIV-1 gene expression.

Structure of the PCAF Bromodomain/Tat Peptide Complex

To understand the structural basis of this molecular recognition, we determined the three-dimensional structure of the PCAF bromodomain in complex with Tat AcK50 peptide from a total of 2903 NMR-derived restraints. The structure for the protein (residues 723–830) and the peptide (residues 47–54) complex was well defined by the NMR data (Figure 2A, Table 1). The structure of the bromodomain when complexed to the Tat peptide consists of a left-handed four-helix bundle (helices α_Z, α_A, α_B, and α_C) and is similar to its free form structure (Dhalluin et al., 1999), except for the ZA and BC loops that comprise the acetyl-lysine binding site and undergo local conformational changes to accommodate peptide binding (see below). The Tat peptide adopts an extended conformation and lies across a pocket formed between the ZA and BC loops (Figure 2B). The side chain of the acetyl-lysine intercalates into the protein hydrophobic cavity and interacts extensively with residues F748, V752, Y760, I764, Y802, and Y809 (Figure 2C). Peptide residues flanking the acetyl-lysine contact the protein. Particularly, G(AcK$-$2), R(AcK$-$1), and R(AcK$+$3) showed intermolecular NOEs to the protein, and extensive interactions were observed between the side chains of Y(AcK$-$3) and V763 and between Q(AcK$+$4) and E756. These specific interactions confer a highly selective association between PCAF bromodomain and Tat.

Structural comparison of PCAF bromomdomain in the free and ligand-bound forms reveals that structural changes of the protein, largely localized in the ZA and BC loops, are directly coupled with the peptide binding (Figure 2D). These structural changes are supported by extensive NMR data, which include changes of chemical shifts and NOE patterns for the backbone amides, side chain methyl groups, and aromatic rings of many protein

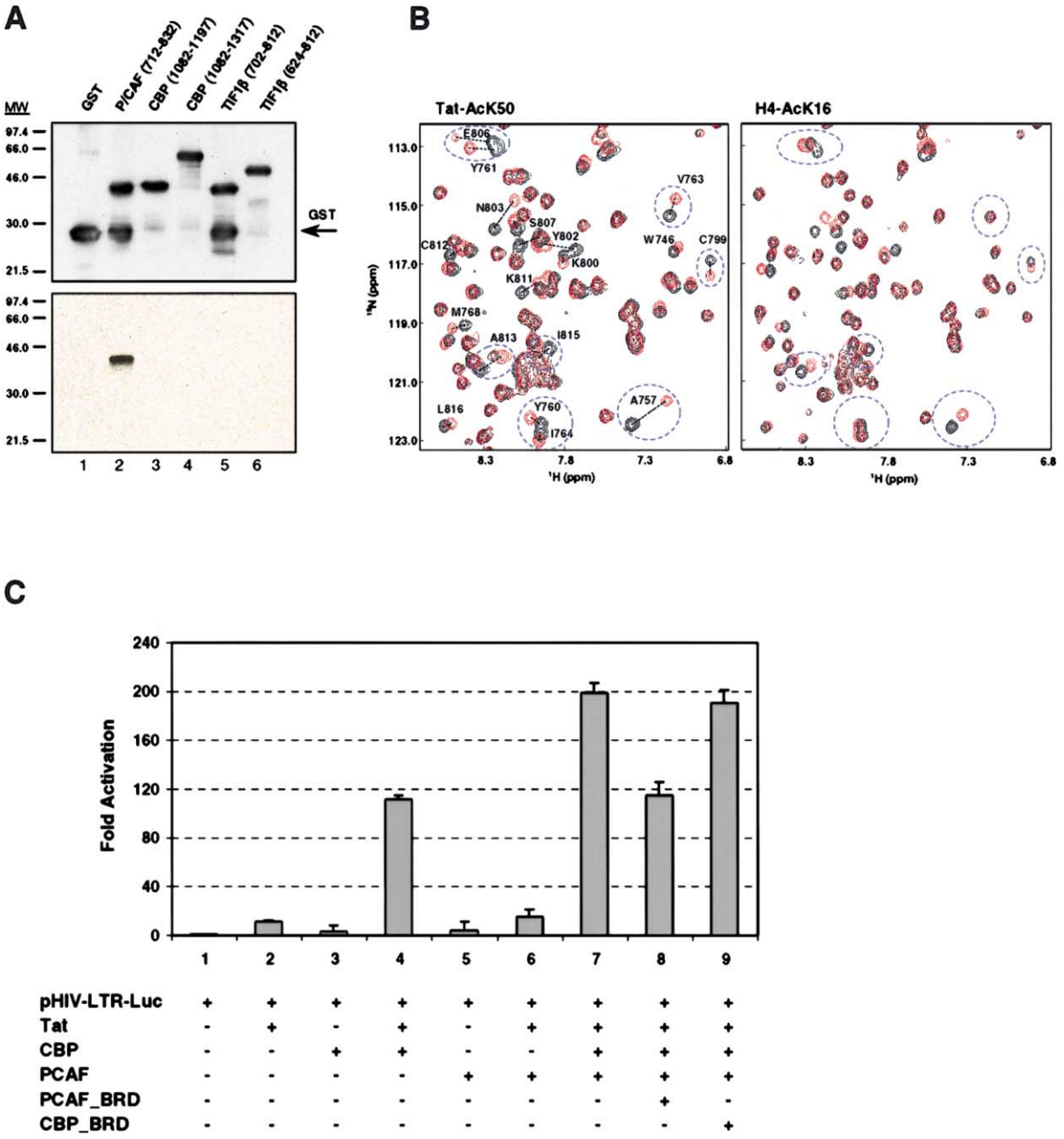

Figure 1. Recognition of Lysine-Acetylated HIV-1 Tat by PCAF Bromodomain

(A) Binding of bromodomains alone from PCAF, CBP, and TIF1β or in combination with PHD fingers from CBP and TIF1β to a biotinylated and lysine-acetylated Tat AcK50 peptide immobilized on streptavidin agarose beads. The upper panel shows purity of the GST-fusion bromodomains used in the assay, separated by SDS-PAGE and stained with Coomassie blue. The lower panel depicts Western blot with anti-GST antibodies, showing specific interaction between PCAF bromodomain and Tat AcK50 peptide.

(B) Comparison of PCAF bromodomain binding to lysine-acetylated peptide derived from HIV-1 Tat at K50 (SYGR-AcK-KRRQR) (left) versus one derived from histone H4 at K16 (SGRGKGGKGLGKGGA-AcK-RHRK) (right). The protein samples were completely titrated with the lysine-acetylated peptide of Tat or H4 with molar ratio 1:1.5 or 1:6, respectively. The 2D ^{1}H-^{15}N HSQC spectra of the bromodomain show changes of backbone amide resonances of the protein in the presence (red) or absence (black) of the peptide ligand. Blue dashed cycles highlight protein residues that exhibited major chemical shift perturbations upon Tat AcK50 peptide binding (left), significantly greater than those observed upon addition of the histone H4 peptide (right).

(C) Functional contribution of PCAF bromodomain and Tat interaction to synergistic stimulation of Tat transcriptional activity by PCAF and CBP. The plasmids used in various combinations in transfections with human 293T cells are as indicated below the graph. The amounts of the plasmids used in transfection experiments are pHIV-LTR-Luc (100 ng), pcTat (100 ng), pRSV-HA-CBP (2.0 ug), pCI-FLAG-PCAF (2.0 ug), pCMV-HA-PCAF_BRD (0.5 ug), and pCMV-FLAG-CBP_BRD (0.5 ug). Total amounts of DNA for transfections were kept constant with addition of empty control vector. Luciferase activities of the cell cytoplasmic extracts were measured using a luciferase-based assay (Promega) 24 hr after transfection and normalized to the β-galactosidase plasmid uptake as described in the Experimental Procedures. Fold activation in 293T cells is expressed relative to the basal expression of pHIV-LTR-Luc set as 1. Mean values of the luciferase activities represent at least three independent transfection experiments.

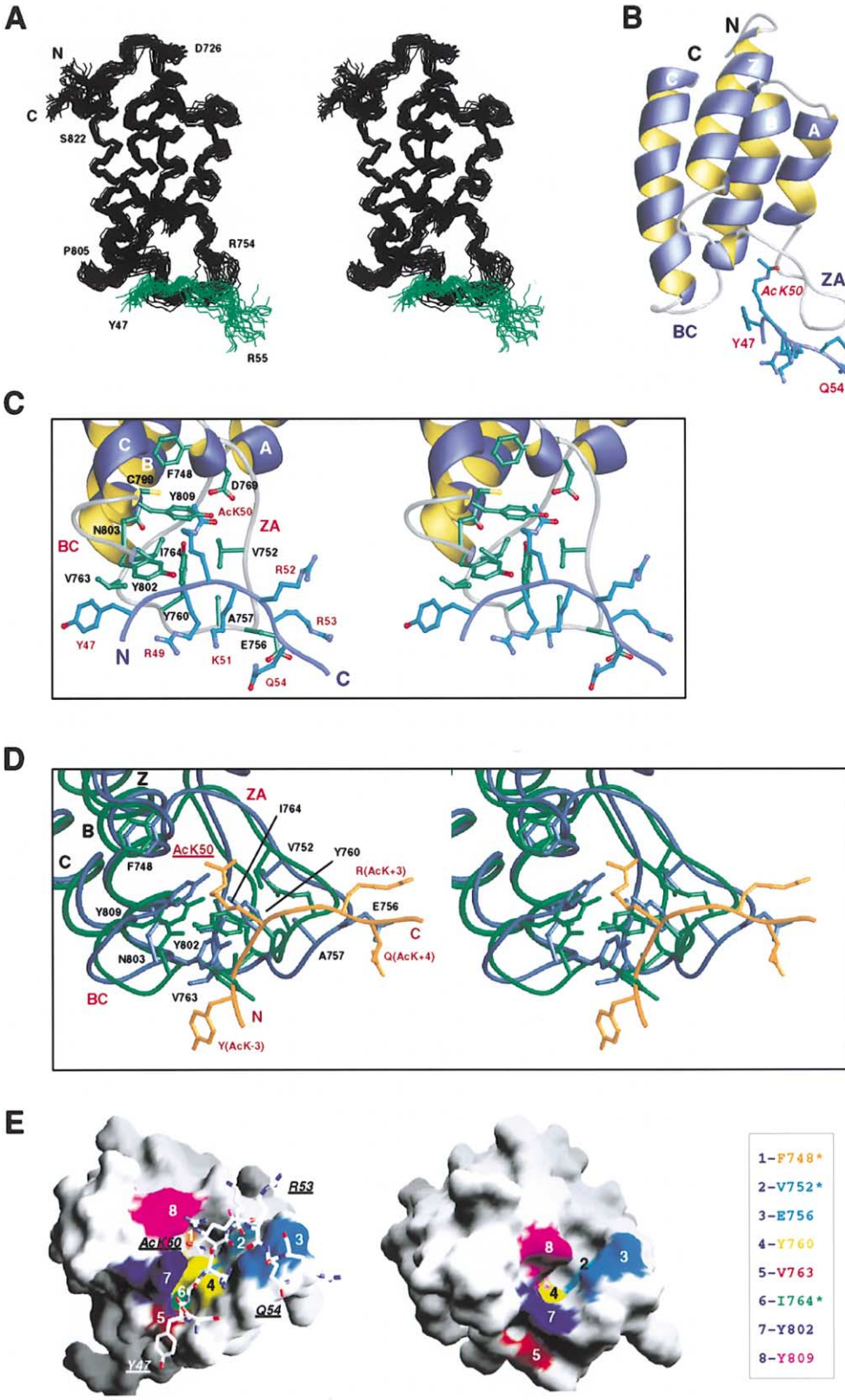

Figure 2. NMR Structure of the PCAF Bromodomain/Tat AcK50 Peptide Complex

(A) Stereoview of the backbone atoms (N, Cα, and C′) of 25 superimposed NMR-derived structures of the PCAF bromodomain (black) (showing residues 719–830) in complex with the Tat AcK50 peptide (green) (showing residues 46–55). Note that amino acid residues in the Tat peptide are described either according to their relative positions with respect to the acetyl-lysine in the sequence or for clarity numbered by their specific positions in the protein sequence of Tat.

Table 1. Summary of NMR Structural Statistics

Total Experimental Restraints	2903	
Total NOE Distance Restraints[a]	2822	
Protein		
Total ambiguous	122	
Total unambiguous	2590	
Intraresidue	1093	
Interresidue		
Sequential ($\|i - j\| = 1$)	480	
Medium ($2 \leq \|i - j\| \leq 4$)	547	
Long range ($\|i - j\| > 4$)	470	
Peptide	32	
Intermolecular	78	
Hydrogen Bond Restraints	28	
Dihedral Angle Restraints	53	
Final Energies (kcal·mol^{-1})		
E_{Total}	366.4 ± 31.1	
E_{NOE}	58.0 ± 12.6	
$E_{Dihedral}$	0.6 ± 0.3	
E_{L-J}[b]	−569.5 ± 22.4	
	Protein/Peptide Complex[f]	Secondary Structure
Ramachandran Plot (%)		
Most favorable region	72.1 ± 2.3	92.0 ± 3.0
Additionally allowed region	22.9 ± 2.4	7.4 ± 3.1
Generously allowed region	3.6 ± 1.4	0.6 ± 0.1
Disallowed region	1.3 ± 0.6	0.0 ± 0.0
Cartesian coordinate RMSDs (Å)[c]		
Backbone atoms (N, Cα, and C′)[d]	0.66 ± 0.14	0.39 ± 0.05
Heavy atoms[d]	1.25 ± 0.18	0.96 ± 0.08
Backbone atoms (N, Cα, and C′)[e]	0.50 ± 0.16	
Heavy atoms[e]	1.83 ± 0.50	
Backbone atoms (N, Cα, and C′)[f]	0.72 ± 0.15	0.54 ± 0.09
Heavy atoms[f]	1.39 ± 0.20	1.25 ± 0.16

[a] Of the total 2822 NOE-derived distance restraints, 341 were obtained by using ARIA program, of which 122 are classified as ambiguous NOEs. The latter NOE signals in the NMR spectra match with more than one proton atom in both the chemical shift assignment and the final NMR structures.
[b] The Lennard-Jones potential was not used during any refinement stage.
[c] None of these final structures exhibit NOE-derived distance restraint violations greater than 0.3 Å or dihedral angle restraint violations greater than 5°.
[d] Protein residues 723–830.
[e] Peptide residues 47–52 and 53–54.
[f] Protein residues 723–830 and peptide residues 47–52 and 53–54.

residues (Figure 1B; see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/9/3/575/DC1). For instance, aromatic protons of Y802 in the BC loop and Y760 in the ZA loop show numerous long-range NOEs in the free form, which become completely absent in the peptide-bound form (see Supplemental Figures S2A, S2B, and S2C at http://www.molecule.org/cgi/content/full/9/3/575/DC1). These changes of NOE patterns are reflected in a ~90° rotational flip of the aromatic ring of Y802, which opens a channel lined by the ZA and BC loops to grasp the peptide through intermolecular interactions such as those observed between Y(AcK-3) and V763 (Figure 2D). Changes of loop conformation in the ZA and BC loops also result in exposing otherwise almost completely buried protein residues such as F748, V752, and I764 for direct peptide recognition (Figure 2E). Supporting NMR data include: (1) the methyl group (δ1) of I764 in the ZA loop shows a NOE cross peak to H^{α} of Y802, only in the free but not in the complex form; and (2) the methyl group of A757 in the ZA loop changes its spatial position from being close to the aromatic ϵ protons of Y802 (Y802.ϵ) in the free form to being proximal to Y761.ϵ upon binding to the Tat peptide (see Supplemental Figures S2D and S2E at

(B) Ribbons (Carson, 1991) representation of the average minimized NMR structure of the PCAF bromodomain/Tat peptide complex.
(C) Stereoview of the Tat binding site in the bromodomain showing side chains of the protein (green) and peptide (blue) residues that are directly involved intermolecular interactions.
(D) Stereoview of superimposition of the free (green) and ligand-bound (blue) structures of PCAF bromodomain showing side chain conformation of the residues in the Tat peptide binding site. The residues of the Tat peptide are colored in orange.
(E) Surface representation of the Tat binding site of the bromodomain in ligand-bound (left) and free form (right). Protein residues important in ligand recognition are colored with the same color scheme in both structures. Residues indicated by an asterisk are almost completely buried in the free form structure.

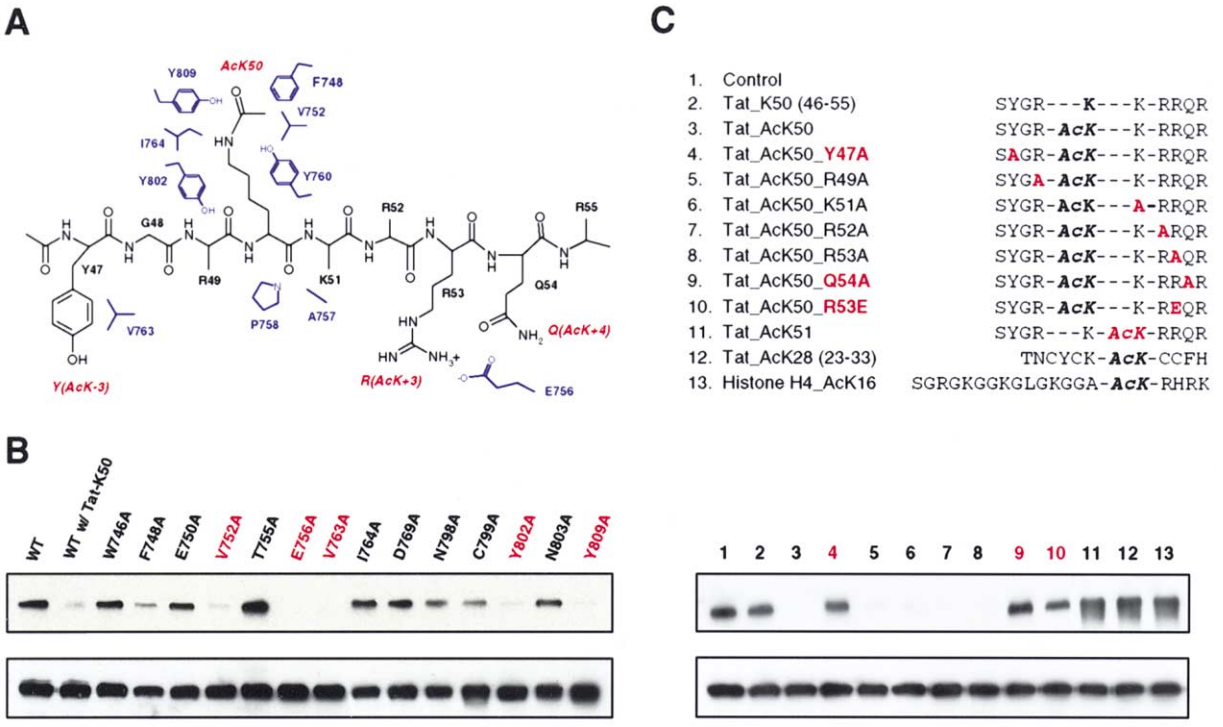

Figure 3. Mutational Analyses of PCAF Bromodomain Binding to HIV-1 Tat

(A) Schematic diagram showing amino acid residues involved in the protein/peptide interface.

(B) Effect of point mutation of protein residues on Tat AcK50 peptide binding. Western blot with anti-GST antibodies shows binding of the GST-fusion PCAF bromodomain proteins to the biotinylated Tat AcK50 peptide immobilized on streptavidin agarose beads (upper panel). The lower panel indicates a relatively equal amount of bromodomain proteins used in each binding experiment. Protein residues highlighted in red exhibited a significant reduction in binding to the Tat AcK50 peptide due to an alanine substitution.

(C) Mutational analysis of Tat peptide residues. Mutation effect was assessed by a peptide competition assay using anti-GST Western blot, in which a nonbiotinylated peptide competes with the biotinylated wild-type Tat AcK50 peptide for binding to the GST-fusion PCAF bromodomain. Numerals above the upper panel indicate a specific peptide used in the competition assay. The numerals in red refer to mutant Tat peptides that showed a significant reduction in binding to the bromodomain in the competition assay. The lower panel shows the relatively equal amount of bromodomain proteins used in each study. For clarity, the peptide residues are numbered according to their positions in the Tat protein sequence.

http://www.molecule.org/cgi/content/full/9/3/575/DC1). Together, our NMR data strongly suggest that conformational changes of protein residues in the ligand binding site are directly coupled with the highly selective interactions between PCAF bromodomain and acetylated Tat.

Specificity of PCAF Bromodomain and Tat Recognition

To determine the relative contributions of bromodomain residues in Tat binding site (Figure 3A), we examined its mutant proteins for binding to an N-terminal biotinylated Tat AcK50 peptide immobilized onto streptavidin agarose beads. Mutation of bromodomain residues W746, E750, T755, I764, D769, N798, C799, or N803 to alanine did not affect peptide binding, whereas proteins containing an alanine mutation at F748, V752, Y802, or Y809 showed a major reduction or nearly complete loss in Tat binding (Figure 3B). Moreover, alanine substitution of V763 or E756 almost completely abrogated peptide association, underlining the importance of their specific interactions with peptide residues Y(AcK−3) and Q(AcK+4). It is important to note that since both V763 and E756 are solvent exposed and located in loop regions of the structure (Figure 2D), their individual mutation to alanine unlikely causes major conformational disruption of the protein. Mutation results of these protein residues are consistent with the NMR structure of the complex and confirm their direct interactions with the acetyl-lysine and/or its flanking residues in the Tat peptide.

To further identify determinants in Tat sequence for PCAF bromodomain recognition, we synthesized mutant peptides and tested their binding to the protein in a peptide competition assay. Because of the high sensitivity of this detection method, the binding study was performed at a protein concentration (10 μM) much lower than that required for NMR study (~200 μM), ensuring specificity of protein-peptide interactions. As anticipated, lysine-acetylated peptides derived from Tat at K51 or K28 (lanes 11 and 12 in Figure 3C) or from histone H4 at K16 (lane 13) showed almost no competition against Tat AcK50 peptide in PCAF bromodomain binding, demonstrating that the latter interaction is of high affinity and specificity. Alanine substitution of resi-

dues R(AcK−1), K(AcK+1), R(AcK+2), or R(AcK+3) in Tat AcK50 peptide slightly weakened its binding to the bromodomain. Conversely, change of Y(AcK−3) (lane 4) or Q(AcK+4) (lane 9) to alanine caused a nearly complete loss of bromodomain binding, confirming the importance of their pairwise interactions with V763 and E756 for Tat-PCAF association. Finally, while mutation of R(AcK+3) to alanine (lane 8) did not significantly alter Tat binding to the bromodomain, its substitution to glutamic acid (lane 10) exhibited a marked reduction in the protein/peptide interaction. The effect of the latter mutation is likely due to an electrostatic repulsion between the glutamate and E756 of the protein. Together, these results explain the structural basis for the highly selective nature of PCAF and lysine-acetylated Tat association, which requires specific interactions of the bromodomain with AcK50 and its flanking residues, including Y(AcK−3), R(AcK+3), and Q(AcK+4).

PCAF Bromodomain Competing against TAR RNA for Binding to Lysine-Acetylated Tat

The arginine-rich motif containing R52 and R53 in Tat is also known to interact with the HIV-1 TAR RNA element (Aboul-ela et al., 1995; Long and Crothers, 1999; Rana and Jeang, 1999). Tat acetylation at K50 by p300/CBP promotes Tat dissociation from TAR RNA in early transcriptional elongation (Deng et al., 2000; Kiernan et al., 1999). To determine whether lysine acetylation directly affects Tat association with TAR RNA, we performed an NMR study of a 27 nucleotide HIV-1 TAR RNA binding to Tat peptides containing either K50 or AcK50. Our results showed that TAR RNA bound to the nonacetylated Tat peptide with a subnanomolar affinity (K_D), in agreement with results reported previously (Aboul-ela et al., 1995; Long and Crothers, 1999), and that K50 acetylation of Tat resulted in a significant reduction of its affinity to TAR RNA (data not shown). More strikingly, we found that PCAF bromodomain competes effectively against TAR RNA for binding to Tat AcK50 peptide (Figures 4A and 4B), suggesting that the binding affinity (K_D) of the latter interaction is on the order of low micromolar. This observation may be explained by possible conformational change of the peptide residues due to acetylation at K50 or involvement of R53 of Tat in both interactions. These results strongly imply that the PCAF bromodomain interaction with AcK50 on Tat not only contributes to Tat-PCAF association but also to the release of lysine-acetylated Tat from TAR RNA association, leading to Tat-mediated HIV-1 transcriptional activation.

Differences of Ligand Selectivity of Bromodomains

Structural comparison of bromodomains from PCAF and other proteins extends our understanding of differences in ligand selectivity. Recent structures of bromodomains from human GCN5 (Hudson et al., 2000) and *Saccharomyces cerevisiae* GCN5p (Owen et al., 2000), and the double bromodomain module of human $TAF_{II}250$ (Jacobson et al., 2000), reinforce the notion that the left-handed four-helix bundle fold of the PCAF bromdomain is conserved in the bromodomain family (Dhalluin et al., 1999). Structural similarity is high for the four helices with pairwise root-mean-square deviations of 0.7–1.8 Å for the backbone Cα atoms. The majority of structural deviations are localized in the loop regions, particularly in the ZA and BC loops (see Supplemental Figure S3 at http://www.molecule.org/cgi/content/full/9/3/575/DC1).

The crystal structure of scGCN5p bromodomain solved in complex with an acetylated peptide derived from histone H4 at K16 (A-AcK-RHRKILRNSIQGI) reveals that the mechanism of acetyl-lysine recognition is highly conserved in bromodomains—it involves a nearly identical set of corresponding conserved residues in the PCAF and scGCN5p bromodomains (Figures 5A and 5B) (Owen et al., 2000). In addition to the acetyl-lysine, scGCN5p bromodomain has a limited number of contacts with two residues at (AcK+2) and (AcK+3) in the H4 peptide. Binding of H(AcK+2) to aromatic rings of Y406 and F367 in scGCN5p is reminiscent of PCAF bromodomain recognition of Tat Y(AcK−3) through interactions with Y802 and V763, which are equivalent to the two scGCN5p residues. Because of this similar mode of molecular interaction, the two aromatic residues in the Tat and H4 peptides, which are located in very different positions with respect to the acetyl-lysine, are bound in a nearly identical position in the corresponding bromodomain structures (Figure 5A). High conservation of these residues in bromodomains (Figure 5B) suggests that selection of an aromatic or hydrophobic residue neighboring the acetyl-lysine is possibly conserved for many members of the bromodomain family.

It is important to note that while the major binding determinant in scGCN5p bromodomain-H4 complex is the acetyl-lysine (Owen et al., 2000), the highly specific association of PCAF bromodomain and Tat peptide is dependent on its interactions not only with the acetyl-lysine and Y(AcK−3) but also with residues on the other side of the acetyl-lysine at (AcK+3) and (AcK+4) (Figures 3B and 3C). These differences in the extent of ligand interactions explain why the Tat AcK50 peptide competes effectively against a similar histone H4 AcK16 peptide for binding to the PCAF bromodomain (Figure 3C, lane 13). Moreover, these differences in ligand selectivity provide an explanation for the striking differences in location and orientation of the bound peptides in the two bromodomains—the backbones of the Tat and H4 peptides lie in the two corresponding structures nearly antiparallel to each other (Figure 5A). Binding of A757 and E756 in the ZA loop to R(AcK+3) and Q(AcK+4) of the Tat peptide, which are completely lacking in the scGCN5 bromodomain-H4 complex, further explains why the PCAF bromodomain undergoes more extensive conformational changes in the ligand site than those seen in the GCN5 bromodomains (see Supplemental Figure S3 at http://www.molecule.org/cgi/content/full/9/3/575/DC1). While the biological relevance of the scGCN5 and histone H4 AcK16 interaction remains to be determined, a growing body of evidence, including previous reports (Benkirane et al., 1998; Deng et al., 2000), our present study of NMR structure and in vitro mutagenesis, and results from in vivo functional studies of Tat-mediated HIV-1 transcriptional activation (Figure 1C and M.O. and E.V., unpublished data), strongly support the biological relevance and importance for the highly selective association of PCAF bromodomain and acetylated Tat.

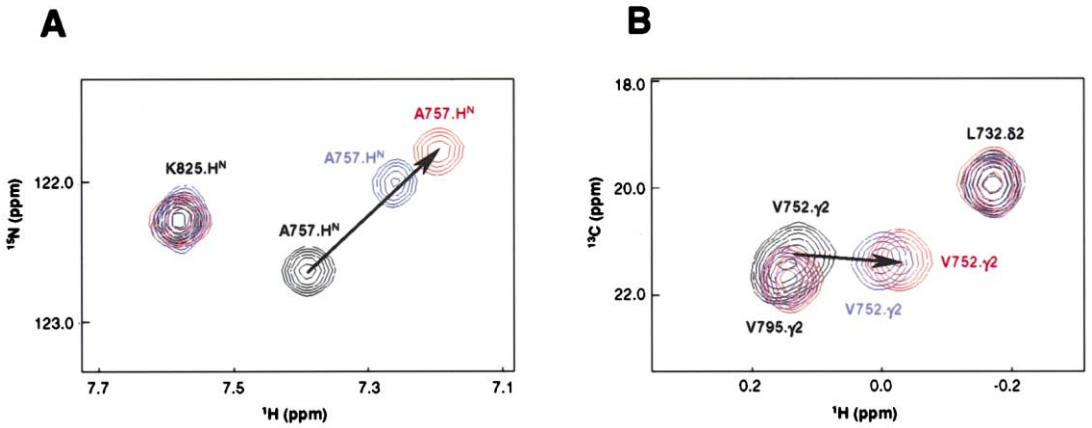

Figure 4. PCAF Bromodomain Competing against TAR RNA for Binding to Tat AcK50 Peptide

(A) Superimposition of a selected region of 2D ^{1}H-^{15}N HSQC spectra of a $^{13}C/^{15}N$-labeled PCAF bromodomain protein in the free form (black), in the presence of Tat AcK50 peptide (molar ratio of 1:1.2) (red), and in the presence of Tat AcK50 peptide and TAR RNA (molar ratio of 1:1.2:1 for protein:peptide:RNA) (blue). The spectra show chemical shift changes of the backbone amide resonances of protein residues due to peptide binding.

(B) Superimposition of 2D ^{1}H-^{13}C HSQC spectra of the PCAF bromodomain collected under the same conditions as described in (A). The NMR spectra exhibit chemical shift changes of the side chain methyl group resonances of protein residues due to peptide binding. The same color-coding scheme was used as in (A). Arrows indicate chemical shift changes of protein NMR resonances from the free form (black) to the Tat AcK50 peptide-bound form (red). Note that only the bromodomain residues (i.e., A757 and V752) that directly interact with the Tat peptide, as shown in the three-dimensional structure, exhibited major chemical shift changes upon peptide binding or in competing against TAR RNA for binding to the Tat peptide. More importantly, addition of TAR RNA causes only small shifts of the protein signals from the Tat peptide-bound position toward the free form position, suggesting that the PCAF bromodomain competes effectively against TAR RNA for binding to the lysine-acetylated Tat peptide. We observed by NMR no significant nonspecific interactions between the protein and TAR RNA under these conditions.

Since bromodomain residues important for acetyl-lysine recognition are largely conserved, binding of acetyl-lysine on a protein is likely a general biochemical function for bromodomains. However, differences in ligand selectivity may be attributed to a few but important differences in bromodomain sequences (Figure 5B), which include (1) variations in the ZA loop such as relatively low sequence conservation and amino acid deletion or insertion; and (2) variation of bromodomain residues that are involved in direct interactions with residues surrounding acetyl-lysine in a target protein. For instance, E756 in the bromodomain of PCAF is unique and only present in a small subset of bromodomains including GCN5. An analogous residue in the structurally similar bromodomain of CBP or p300 (Y.H. and M.-M.Z., unpublished data) is a leucine followed by a 2 amino acid insertion, which are present in a small subfamily of bromodomains (Figure 5B) (Jeanmougin et al., 1997). Moreover, a short helix corresponding to the AWPFM sequence in the ZA loop of PCAF (residues 745–749) is likely completely missing in TIF1β bromodomain due to amino acid deletion, and E756 of PCAF is substituted with a two residue AT motif in the sequence. Together, these findings explain why bromodomains from CBP and TIF1β did not interact with Tat AcK50 peptide (Figure 1A).

Human GCN5-S, a shorter version of hGCN5 that contains only the HAT domain and the bromodomain but lacks the N-terminal p300/CBP binding domain due to an alternative RNA splicing (Schiltz and Nakatani, 2000; Yamauchi et al., 2000), has been recently reported to interact with HIV Tat in vitro (Col et al., 2001). This Tat interaction involves both the HAT domain and the bromodomain of hGCN5-S. While the specific binding site on Tat by the hGCN5 bromodomain and the question of whether this bromodomain interaction is dependent on Tat lysine acetylation remain to be determined, our data reported here suggest that the hGCN5 bromodomain may possess ligand selectivity similar to that of the structurally homologous bromodomain of PCAF.

Conclusion

Tat-stimulated transcriptional activation of integrated HIV-1 genomes defines the rate-limiting step for viral replication (Adams et al., 1994; Benkirane et al., 1998). Tat synergy with histone acetyltransferases and its recruitment of PCAF via a bromodomain interaction, as we described in this study, support the notion that Tat transactivation of HIV-1 chromosomal transcription proceeds via chromatin remodeling (Deng et al., 2000; Kiernan et al., 1999; Ott et al., 1999). Our findings may explain why deletion of the PCAF C-terminal region comprising the bromodomain potently abrogated Tat transactivation of integrated but not unintegrated HIV-1 LTR (Benkirane et al., 1998). Our study reinforces the concept that bromodomain and acetyl-lysine recognition could serve as a pivotal mechanism for controlling protein-protein interactions in chromatin remodeling as well as other cellular processes including viral life cycle (Dyson et al., 2001; Winston and Allis, 1999), and that differences

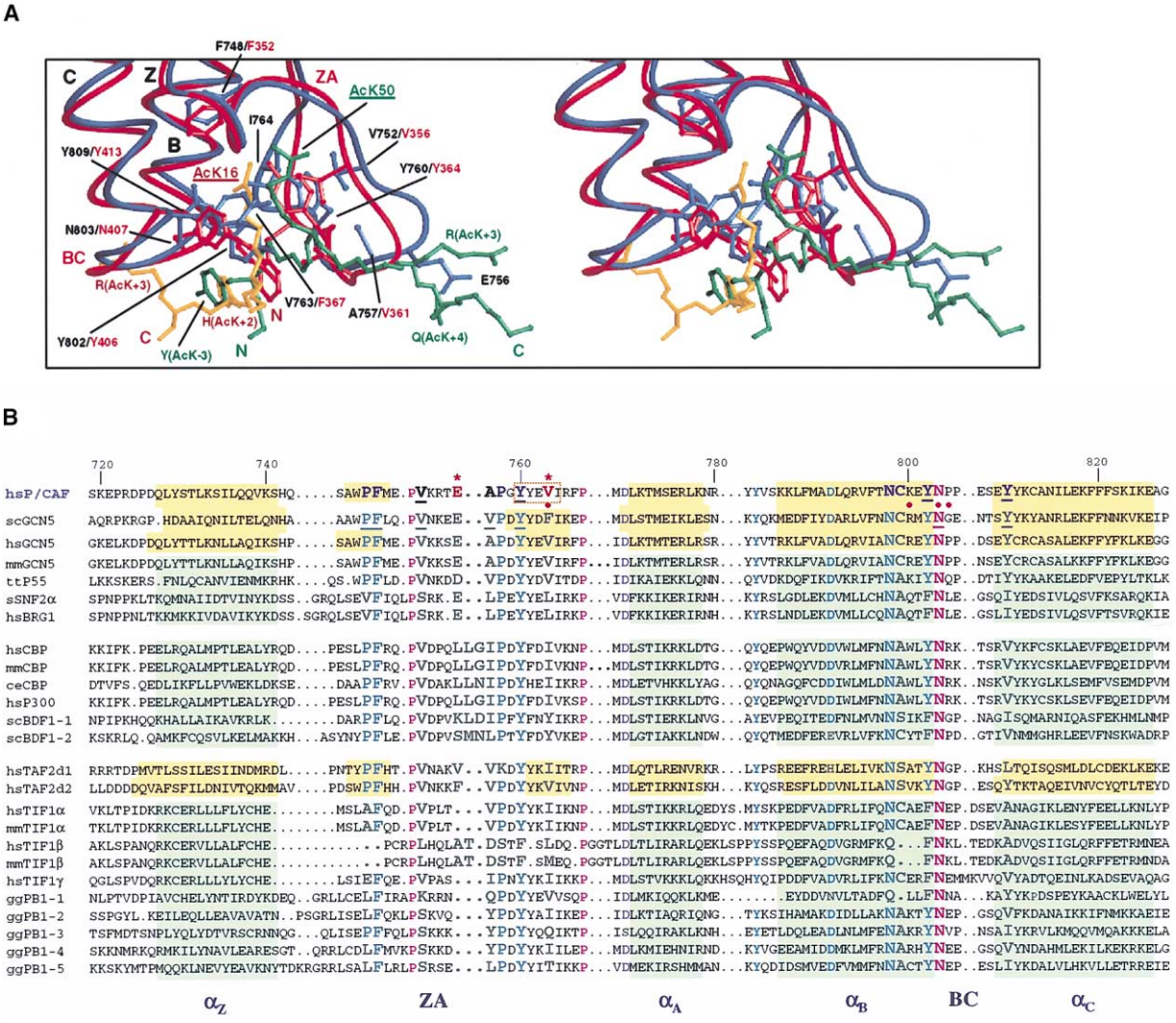

Figure 5. Differences in Ligand Selectivity of Bromodomains

(A) Stereoview of superimposition of the structures of the PCAF bromodomain-Tat peptide complex (blue) and the scGCN5 bromodomain-H4 peptide complex (red) showing conformational differences of protein residues in the peptide binding sites. The lysine-acetylated peptides of HIV-1 Tat and histone H4 are shown in green and orange, respectively. The residues of the PCAF and scGCN5 bromodomains are numbered according to protein sequences and color-coded in black and red, respectively. The corresponding conserved residues in the two bromodomains are annotated together. The amino acid residues of the Tat and H4 peptides are described according to their position with respect to the acetyl-lysine in the corresponding peptide sequences. The structures were superimposed on the four helices of the two bromodomains in Insight and the figure was prepared with Ribbons.

(B) Sequence alignment of a selected number of bromodomains. The sequences were aligned based on the experimentally determined three-dimensional structures of five bromodomains, highlighted in yellow. Note that in the PCAF bromodomain, a short helix in the ZA loop comprising the YYEVI sequence (residues 760–764) (boxed by dashed lines) was only observed in the free form but not in the Tat peptide-bound form. The predicted secondary structures in the corresponding regions of other bromodomains are shown in green. Because of relatively high variations in amino acid sequence in the ZA loop, prediction of secondary structures was omitted. Bromodomains are grouped on the basis of the predicted sequence and/or structural similarities. Residue numbers of the PCAF bromodomain are indicated above its sequence. Three absolutely conserved residues, corresponding to P751, P767, and N803 in the PCAF bromodomain, are shown in purple. Highly conserved residues are colored in blue. The residues in the PCAF bromodomain that directly interact with the Tat peptide, as determined by intermolecular NOEs, are displayed in a larger font size. The residues essential for the acetyl-lysine binding are underlined, and the residues important for ligand selectivity through interactions with the peptide residues flanking the acetyl-lysine are highlighted by red asterisks. The protein residues contacting the acetyl-lysine in the scGCN5 bromodomain-H4 peptide complex are underlined, and residues contacting other parts of the peptide are indicated by red dots.

in ligand selectivity of conserved protein modular domains can be achieved by evolutionary changes of amino acid sequences in the ligand binding site. The new knowledge of the structural basis of PCAF bromodomain and Tat recognition should aid in the design of small molecules that can be used to block this specific

interaction in order to disrupt HIV-1 transcriptional activation and replication.

Experimental Procedures

Sample Preparation

The PCAF bromodomain (residues 719–832) was expressed in *Escherichia coli* BL21(DE3) cells using the pET14b vector (Novagen) (Dhalluin et al., 1999). Isotope-labeled bromodomain proteins were prepared from cells grown on a minimal medium containing $^{15}NH_4Cl$ with or without $^{13}C_6$-glucose in either H_2O or 75% ^2H_2O. The protein was purified by affinity chromatography on a nickel-IDA column (Invitrogen), followed by the removal of poly-His tag by thrombin cleavage. GST-fusion bromodomains from PCAF, CBP, and TIF1β were expressed in *E. coli* BL21 (DE3) codon plus cells using the pGEX4T-3 vector (Pharmacia) and purified with a glutathione sepharose column. NMR spectra of the recombinant CBP and TIF1β proteins were acquired to ensure that they were properly folded and functional (see Supplemental Figure S4 at http://www.molecule.org/cgi/content/full/9/3/575/DC1). The acetyl-lysine-containing peptides were prepared on a MilliGen 9050 peptide synthesizer (Perkin Elmer) using Fmoc/HBTU chemistry. Acetyl-lysine was incorporated using the reagent Fmoc-Ac-Lys with HBTU/DIPEA activation. The HIV-1 TAR RNA was obtained from Dharmacon Research, Inc. (Lafayette, CO).

NMR Spectroscopy

NMR samples contained a protein/peptide complex of 0.5 mM in 100 mM phosphate buffer (pH 6.5) containing 5 mM perdeuterated DTT and 0.5 mM EDTA in $H_2O/^2H_2O$ (9/1) or ^2H_2O. All NMR spectra were acquired at 30°C on a Bruker 500 or 600 MHz NMR spectrometer. The ^1H, ^{13}C, and ^{15}N resonances of the protein backbone and side chain atoms were assigned by using a standard set of triple-resonance experiments (Sattler et al., 1999) with a uniformly $^{13}C/^{15}N$-labeled and 75% deuterated protein in complex with an unlabeled peptide. The distance restraints were obtained from ^{13}C- or ^{15}N-edited three-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) spectra (Clore and Gronenborn, 1994). ϕ-angle restraints were determined based on the $^3J_{HN,H\alpha}$ coupling constants measured in a 3D HNHA spectrum (Clore and Gronenborn, 1994). Slowly exchanging amide protons were identified from a series of 2D ^{15}N-HSQC spectra recorded after the H_2O buffer was changed to a ^2H_2O buffer, which were used together with the initial structures calculated with only NOE-derived distance restraints to generate hydrogen-bond distance restraints in final structure calculations. The intermolecular NOEs were detected in ^{13}C-edited (F_1), $^{13}C/^{15}N$-filtered (F_3) 3D NOESY spectrum (Clore and Gronenborn, 1994). All NMR spectra were processed with the NMRPipe program (Delaglio et al., 1995) and analyzed using NMRView (Johnson and Blevins, 1994). NMR binding studies of Tat peptides and TAR RNA interactions were performed in the phosphate buffer (pH 6.5) containing 200 mM NaCl to minimize any nonspecific interactions.

Structure Calculations

Structures of the protein/peptide complex were calculated with a distance geometry-simulated annealing protocol using the X-PLOR program (Brunger, 1993). A total of 2359 manually assigned NOE-derived distance restraints were used in initial structure calculations. The ARIA (Nilges and O'Donoghue, 1998)-assigned distance restraints agree with the structures calculated using only the manually determined NOE distance restraints, 28 hydrogen-bond distance restraints, and 53 ϕ angle restraints. The final structure calculations employed a total of 2903 NMR experimental restraints from the manual and the ARIA-assisted assignments, including 2700 unambiguous intramolecular and 78 intermolecular NOE distance restraints. The distance restraint force constant was 50 kcal mol^{-1} $Å^{-2}$, and no NOE was violated by more than 0.3 Å. The torsion restraint force constant was 200 kcal mol^{-1} rad^{-2}, and no dihedral angle restraint was violated by more than 5°. While only the covalent geometry terms, NOE, torsion, and repulsive van der Waals terms were used in the structure refinement, a large, negative Lennard-Jones potential energy was observed (-569.5 ± 22.4 kcal mol^{-1}), indicating good nonbonded geometry of the structure. Procheck (Laskowski et al., 1996) analysis indicated that over 98% of the protein and peptide residues are in allowed regions of the Ramachandran map.

Mutational Analysis

Site-directed mutant proteins of PCAF bromodomain were prepared with the QuickChange kit (Stratagene). DNA sequencing confirmed the desired mutations. The GST-fusion bromodomains (10 μM) of PCAF, CBP, or TIF1β were incubated with an N-terminal biotinylated and lysine-acetylated Tat peptide (50 μM) in 50 mM Tris buffer (pH 7.5), containing 50 mM NaCl, 0.1% BSA, and 1 mM DTT at 22°C for 2 hr. Streptavidin agarose (10 μL) was added to the mixture, and the beads were washed in the Tris buffer containing 500 mM NaCl and 0.1% NP-40. Proteins eluted from the agarose beads were separated by SDS-PAGE and visualized by Western blotting using anti-GST antibody (Sigma) and horseradish-peroxidase-conjugated goat anti-rabbit IgG. Peptide competition assay was performed by incubating a nonbiotinylated peptide with the PCAF bromodomain and the biotinylated Tat AcK50 peptide. The molar ratio of the former and latter peptides in the mixture was kept at 1:2.

Plasmid Constructs

The mammalian expression vectors for the PCAF and CBP bromodomains were constructed as follows. Coding sequence for the PCAF bromodomain (residues 719–832) was subcloned into EcoRI-XhoI sites of pCMV-HA vector (Clontech). The CBP bromodomain (residues 1082–1197) was subcloned into BamHI-XhoI sites of pCMV-FLAG vector (Stratagene). The expression vectors for the full-length PCAF (pCI-FLAG-PCAF) (Li et al., 2000), the full-length CBP (pRSV-HA-CBP) (Kwok et al., 1996), HIV-1 Tat (pcTat), and the HIV-1 LTR-luciferase reporter construct (pHIV-LTR-Luc) (Bieniasz et al., 1998; Madore and Cullen, 1993) have been previously described.

Cell Culture and Transfections

Human 293T cells were propagated in Dulbecco's modified Eagle's medium with 10% fetal calf serum and transfected using the calcium phosphate coprecipitation method. Amounts of plasmid DNA used in cell transfections are as described in the legend to Figure 1C. The transfected 293T cells were lysed 24 hr after transfection and assayed for luciferase activity of the cell extracts using a luciferase-based assay system (Promega) (Bieniasz et al., 1998; Madore and Cullen, 1993). Luciferase activities derived from HIV-1 LTR were normalized to a cotransfected vector expressing β-galactosidase. The expression level of the transfected proteins was examined by Western blotting using monoclonal antibodies to HA (Roche Diagnostics), FLAG (Stratagene), or β-actin (Sigma), and rabbit polyclonal antibodies to the PCAF bromodomain or the CBP bromodomain (see Supplemental Figure S5 at http://www.molecule.org/cgi/content/full/9/3/575/DC1).

Acknowledgments

We thank P.D. Bieniasz for providing the pcTat and HIV-1 LTR-luc constructs, M.J. Walsh and R.L. Schiltz for the PCAF expression plasmids, and N. Zeleznik-Le for the full-length CBP construct. We also thank I. Wolf for peptide synthesis, C. Dhalluin, O. Plotnikova, and S. Yan for technical advice and support, A. Koch, K. Manzur, and K.S. Yan for critical reading of the manuscript, and A.K. Aggarwal, D.E. Logothetis, and H. Weinstein for helpful suggestions to the study. This work was supported by a National Institutes of Health grant to M.-M. Z.

Received September 18, 2001; revised January 9, 2002.

References

Aasland, R., Gibson, T.J., and Stewart, A.F. (1995). The PHD finger: implications for chromatin-mediated transcriptional regulation. Trends Biochem. Sci. *20*, 56–59.

Aboul-ela, F., Karn, J., and Varani, G. (1995). The structure of the human immunodeficiency virus type-1 TAR RNA reveals principles of RNA recognition by Tat protein. J. Mol. Biol. *253*, 313–332.

Adams, M., Sharmeen, L., Kimpton, J., Romeo, J.M., Garcia, J.V.,

Peterlin, B.M., Groudine, M., and Emerman, M. (1994). Cellular latency in human immunodeficiency virus-infected individuals with high CD4 levels can be detected by the presence of promoter-proximal transcripts. Proc. Natl. Acad. Sci. USA *91*, 3862–3866.

Benkirane, M., Chun, R.F., Xiao, H., Ogryzko, V.V., Howard, B.H., Nakatani, Y., and Jeang, K.-T. (1998). Activation of integrated provirus requires histone acetyltransferase: p300 and P/CAF are co-activators for HIV-1 Tat. J. Biol. Chem. *273*, 24898–24905.

Bieniasz, P.D., Grdina, T.A., Bogerd, H.P., and Cullen, B.R. (1998). Recruitment of a protein complex containing Tat and cyclin T1 to TAR governs the species specificity of HIV-1 Tat. EMBO J. *17*, 7056–7065.

Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carozza, M.J., Tan, S., and Workman, J.L. (2001). Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. Science *292*, 2333–2337.

Brownell, J.E., and Allis, C.D. (1996). Special HATs for special occasions: Linking histone acetylation to chromatin assembly and gene activation. Curr. Opin. Genet. Dev. *6*, 176–184.

Brunger, A.T. (1993). X-PLOR Version 3.1: A System for X-Ray Crystallography and NMR, Version 3.1 edn (New Haven, CT: Yale University Press).

Carson, M. (1991). Ribbons 2.0. J. Appl. Crystallogr. *24*, 958–961.

Clore, G.M., and Gronenborn, A.M. (1994). Multidimensional heteronuclear nuclear magnetic resonance of proteins. Methods Enzymol. *239*, 249–363.

Col, E., Caron, C., Seigneurin-Berny, D., Gracia, J., Favier, A., and Khochbin, S. (2001). The histone acetyltransferase, hGCN5, interacts with and acetylates the HIV transactivator. Tat. J. Biol. Chem. *276*, 28179–28184.

Cullen, B.R. (1998). HIV-1 auxiliary proteins: making connections in a dying cell. Cell *93*, 685–692.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J., and Bax, A. (1995). NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J. Biomol. NMR *6*, 277–293.

Deng, L., de la Fuente, C., Fu, P., Wang, L., Donnelly, R., Wade, J.D., Lambert, P., Li, H., Lee, C.-G., and Kashanchi, F. (2000). Acetylation of HIV-1 Tat by CBP/P300 increases transcription of integrated HIV-1 genome and enhances binding to core histones. Virology *277*, 278–295.

Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.-M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature *399*, 491–496.

Dyson, M.H., Rose, S., and Mahadevan, L.C. (2001). Acetylation-binding and function of bromodomain-containing proteins in chromatin. Front. Biosci. *6*, 853–865.

Friedman, J.R., Fredericks, W.J., Jensen, D.E., Speicher, D.W., Huang, X.P., Neilson, E.G., and Rauscher, F.J., III. (1996). KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes Dev. *10*, 2067–2078.

Garber, M.E., and Jones, K.A. (1999). HIV-1 Tat: coping with negative elongation factors. Curr. Opin. Immunol. *11*, 460–465.

Haynes, S.R., Dollard, C., Winston, F., Beck, S., Trowsdale, J., and Dawid, I.B. (1992). The bromodomain: a conserved sequence found in human, *Drosophila* and yeast proteins. Nucleic Acids Res. *20*, 2603.

Hottiger, M.O., and Nabel, G.J. (1998). Interaction of human immunodeficiency virus type 1 Tat with the transcriptional coactivators p300 and CREB binding protein. J. Virol. *72*, 8252–8256.

Hudson, B.P., Martinez-Yamout, M.A., Dyson, H.J., and Wright, P.E. (2000). Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. J. Mol. Biol. *304*, 355–370.

Jacobson, R.H., Ladurner, A.G., King, D.S., and Tjian, R. (2000). Structure and function of a human TAFII250 double bromodomain module. Science *288*, 1422–1425.

Jeang, K.-T., Xiao, H., and Rich, E.A. (1999). Multifaceted activities of the HIV-1 transactivator of transcription. Tat. J. Biol. Chem. *274*, 28837–28840.

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

Jenuwein, T., and Allis, C.D. (2001). Translating the histone code. Science *293*, 1074–1080.

Johnson, B.A., and Blevins, R.A. (1994). NMRView: a computer program for the visualization and analysis of NMR data. J. Biomol. NMR *4*, 603–614.

Jones, K.A. (1997). Taking a new TAK on Tat transactivation. Genes Dev. *11*, 2593–2599.

Karn, J. (1999). Tackling Tat. J. Mol. Biol. *293*, 235–254.

Keen, N.J., Churcher, M.J., and Karn, J. (1997). Transfer of Tat and release of TAR RNA during the activation of the human immunodeficiency virus type-1 transcription elongation complex. EMBO J. *16*, 5260–5272.

Kiernan, R.E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K.-T., et al. (1999). HIV-1 Tat transcriptional activity is regulated by acetylation. EMBO J. *18*, 6106–6118.

Kouzarides, T. (2000). Acetylation: a regulatory modification to rival phosphorylation? EMBO J. *19*, 1176–1179.

Kwok, R.P., Laurance, M.E., Lundblad, J.R., Goldman, P.S., Shih, H., Connor, L.M., Marriott, S.J., and Goodman, R.H. (1996). Control of cAMP-regulated enhancers by the viral transactivator Tax through CREB and the co-activator CBP. Nature *380*, 642–646.

Laskowski, R.A., Rullmannn, J.A., MacArthur, M.W., Kaptein, R., and Thornton, J.M. (1996). AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J. Biomol. NMR *8*, 477–486.

Li, S.D., Aufiero, B., Schiltz, R.L., and Walsh, M.J. (2000). Regulation of the homeodomain CCAAT displacement/*cut* protein function by histone acetyltransferase p300/CREB-binding protein (CBP)-associated factor and CBP. Proc. Natl. Acad. Sci. USA *97*, 7166–7171.

Long, K.S., and Crothers, D.M. (1999). Characterization of the solution conformations of unbound and Tat peptide-bound forms of HIV-1 TAR RNA. Biochemistry *38*, 10059–10069.

Madore, S.J., and Cullen, B.R. (1993). Genetic analysis of the cofactor requirement for human immunodeficiency virus type 1 Tat function. J. Virol. *67*, 3703–3711.

Manning, E.T., Ikehara, T., Ito, T., Kadonaga, J.T., and Kraus, W.L. (2001). p300 forms a stable, template-committed complex with chromatin: role for the bromodomain. Mol. Cell Biol. *21*, 3876–3887.

Nilges, M., and O'Donoghue, S. (1998). Ambiguous NOEs and automated NOE assignment. Prog. NMR Spectroscopy *32*, 107–139.

Ott, M., Schnolzer, M., Garnica, J., Fischle, W., Emiliani, S., Rackwitz, H.-R., and Verdin, E. (1999). Acetylation of the HIV-1 Tat protein by p300 is important for its transcriptional activity. Curr. Biol. *9*, 1489–1492.

Owen, D.J., Ornaghi, P., Yang, J.C., Lowe, N., Evans, P.R., Ballario, P., Neuhaus, D., Eiletici, P., and Travers, A.A. (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. EMBO J. *19*, 6141–6149.

Rana, T.M., and Jeang, K.-T. (1999). Biochemical and functional interactions between HIV-1 Tat protein and TAR RNA. Arch. Biochem. Biophys. *365*, 175–185.

Sattler, M., Schleucher, J., and Griesinger, C. (1999). Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. Prog. NMR Spectroscopy *34*, 93–158.

Schiltz, R.L., and Nakatani, Y. (2000). The PCAF acetylase complex as a potential tumor suppressor. Biochim. Biophys. Acta *1470*, M37–M53.

Sterner, D.E., Grant, P.A., Roberts, S.M., Duggan, L.J., Belotserkovskaya, R., Pacella, L.A., Winston, F., Workman, J.L., and Berger, S.L. (1999). Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol. Cell. Biol. *19*, 86–98.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

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

Travers, A. (1999). Chromatin modification: how to put a HAT on the histones. Curr. Biol. *9*, 23–25.

Wei, P., Garber, M.E., Fang, S.M., Fischer, W.H., and Jones, K.A. (1998). A novel CDK9-associated C-type cyclin interacts with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. Cell *92*, 451–462.

Winston, F., and Allis, C.D. (1999). The bromodomain: a chromatin-targeting module? Nat. Struct. Biol. *6*, 601–604.

Yamauchi, T., Yamauchi, J., Kuwata, T., Tamura, T., Yamashita, T., Bae, N., Westphal, H., Ozato, K., and Nakatani, Y. (2000). Distinct but overlapping roles of histone acetylase PCAF and of the closely related PCAF-B/GCN5 in mouse embryogenesis. Proc. Natl. Acad. Sci. USA *97*, 11303–11306.

Accession Numbers

Coordinates for the NMR three-dimensional structure of the PCAF bromodomain/HIV-1 Tat peptide complex have been deposited in the Brookhaven Protein Data Bank under the accession code 1JM4.