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Minireview

Bromodomain: an acetyl-lysine binding domain

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Abstract **Bromodomains, an extensive family of evolutionarily conserved protein modules originally found in proteins associated with chromatin and in nearly all nuclear histone acetyltransferases, have been recently discovered to function as acetyl-lysine binding domains. More recent structural studies of bromodomain/peptide ligand complexes have enriched our understanding of differences in ligand selectivity of bromodomains. These new findings demonstrate that bromodomain/acetyl-lysine recognition can serve as a pivotal mechanism for regulating protein–protein interactions in numerous cellular processes including chromatin remodeling and transcriptional activation, and reinforce the concept that functional diversity of a conserved protein modular structure is achieved by evolutionary changes of amino acid sequences in the ligand binding site.** **© 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Bromodomain; Chromatin remodeling; Histone modification; Lysine acetylation; Transcriptional regulation

1. Introduction

The bromodomain, first reported in the *Drosophila* protein brahma (hence the name) [1,2], represents an extensive family of evolutionarily conserved protein modules found in many chromatin-associated proteins and in nearly all known nuclear histone acetyltransferases (HATs) [3]. While it has been long suggested from yeast genetic studies that bromodomains play an important role in chromatin remodeling [4–7], their specific biological functions only began to emerge after the recent discovery that bromodomains function as acetyl-lysine binding domains [8–11]. This new finding suggests a novel mechanism for regulating protein–protein interactions via lysine acetylation, which has broad implications for the mechanisms underlying a wide variety of cellular events, including chromatin remodeling and transcriptional activation [12–14]. Such a mechanism supports the hypothesis that bromodomains can contribute to highly specific histone acetylation by tethering transcriptional HATs to specific chromosomal sites [4,15,16], and to the assembly and activity of multi-protein complexes of chromatin remodeling such as SAGA and NuA4 [17,18]. Moreover, bromodomain/acetyl-lysine recognition may also help understand the underlying molecular mechanisms for a wide range of phenotypes linked to bromodomain deletion. For example, it has been shown that the bromodomain module is indispensable for the function of GCN5 in yeast [19,20]. Deletion of a bromodomain in human HBRM, a protein in the SWI/SNF remodeling complex, causes both decreased stability and loss of nuclear localization [21,22]. Bromodomains of Bdf1, a *Saccharomyces cerevisiae* protein, are required for sporulation and normal mitotic growth [23]. Finally, bromodomain deletion in Sth1, Rsc1 and Rsc2, three members of the nucleosome remodeling complex RSC (remodeling the structure of chromatin), can cause a conditional lethal phenotype (in Sth1) [24] or a strong phenotypic inhibition on cell growth (in Rsc1 and Rsc2) [25]. It is important to note that the phenotypic effect observed in Rsc1 and Rsc2 results from deletion of only the second but not the first bromodomain, suggesting that these two bromodomains serve distinct functions through interactions with different biological ligands [25]. In this minireview, we focus on the recent studies on ligand specificity of bromodomains.

2. The bromodomain structure

The three-dimensional structure of a prototypical bromodomain from the transcriptional co-activator p300/CBP-associated factor (P/CAF) determined by using nuclear magnetic resonance (NMR) spectroscopy showed that the bromodomain adopts an atypical left-handed four-helix bundle (helices α_Z, α_A, α_B and α_C) (Fig. 1A) [8]. A long intervening loop between helices α_Z and α_A (termed the ZA loop) is packed against the loop connecting helices B and C (named the BC loop) to form a surface-accessible hydrophobic pocket, which is located at one end of the four-helix bundle, opposite the amino and carboxy termini of the protein. Mutagenesis studies suggest that tertiary contacts amongst the hydrophobic and aromatic residues between the two inter-helical loops contribute directly to the structural stability of the protein [8].

This unique structural fold is highly conserved in the bromodomain family, as supported by several more recently determined structures of bromodomains from human GCN5 [11] and *S. cerevisiae* GCN5p [10] as well as the double bromodomain module of human $TAF_{II}250$ [9]. The structural similarity amongst these bromodomains is very high for the four helices with pairwise root-mean-square deviations of 0.7–

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Abbreviations: HAT, histone acetyltransferase; HIV-1, human immunodeficiency virus type 1; NMR, nuclear magnetic resonance; P/CAF, p300/CBP-associated factor

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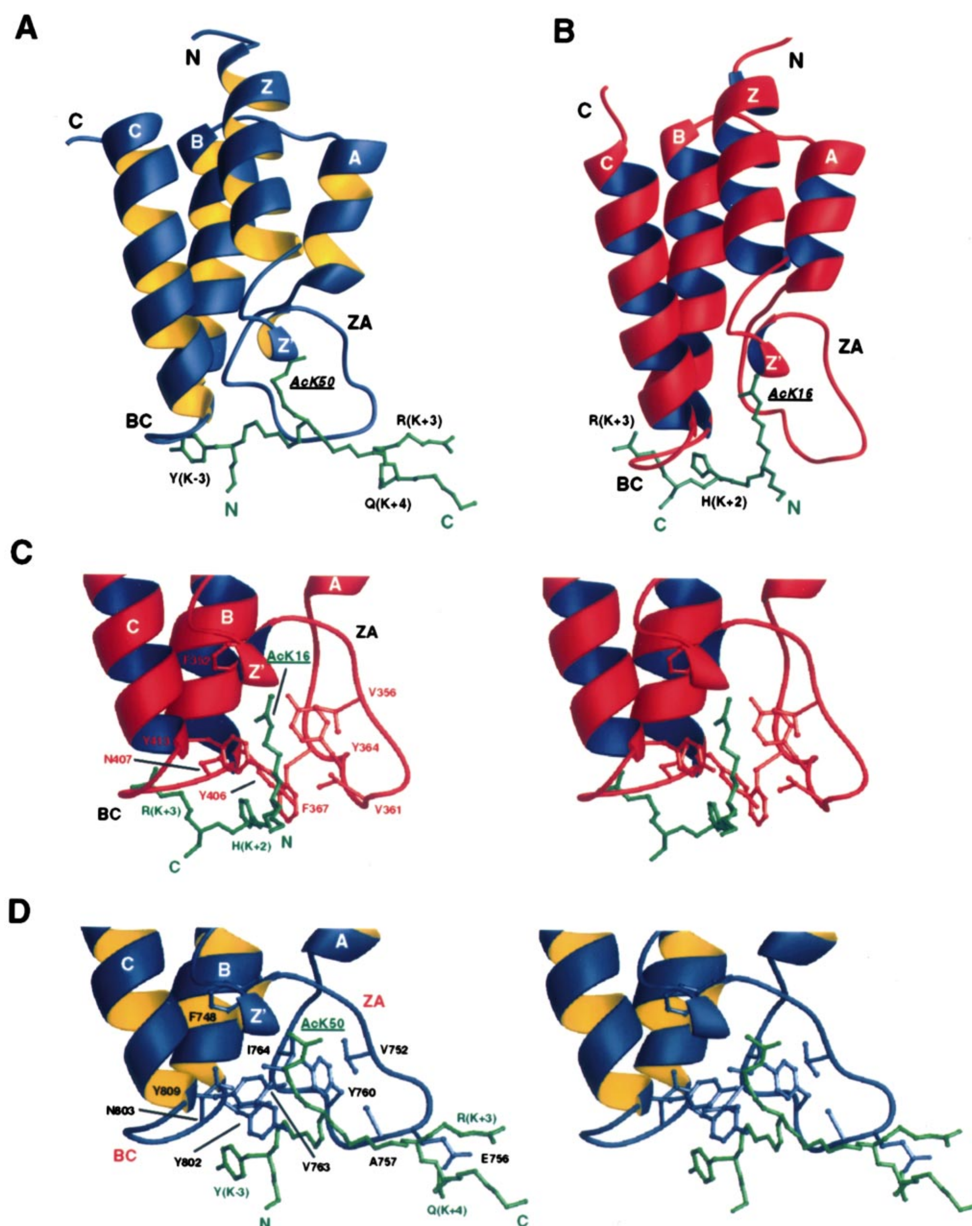

Fig. 1. Differences in ligand selectivity of bromodomains. A: The NMR solution structure of the P/CAF bromodomain (blue) in complex with an lysine-acetylated peptide (green) derived from HIV-1 Tat at K50 (SYGR-AcK-KRRQR). B: The crystal structure of the scGCN5p bromodomain (red) in complex with a histone H4 peptide (green) containing K16 (A-AcK-RHRKILRNSIQGI). C: Stereoview of the scGCN5 bromodomain structure showing interactions of the protein (red) and the H4 peptide (green) residues in the ligand binding sites. D: Stereoview of the P/CAF bromodomain structure showing interactions of the protein (blue) and the Tat peptide (green) residues in the ligand binding sites. The protein residues are numbered according to the sequences, and the peptide residues are annotated according to their positions with respect to the acetyl-lysine.

1.8 Å for the backbone C_α atoms. The majority of structural deviations are localized in the loop regions, particularly in the ZA and BC loops. This observation is in an agreement with the relatively high sequence variations in these loops (Fig. 2) [3]. The modular structure supports the notion that bromodomains act as a functional unit for protein interactions, and multiple bromodomain modules can be placed sequentially in a protein to serve similar or distinct functions [3,8].

3. Bromodomain as an acetyl-lysine binding domain

The discovery of acetyl-lysine recognition by bromodomains is attributed to the unique ability of NMR spectroscopy to measure changes of local chemical environment and/or conformation of a protein induced upon binding to a ligand. Weak but highly specific interactions between a protein and a ligand (with a dissociation constant K_D in the μM–mM range) can thus be detected with NMR. NMR titrations of the P/CAF bromodomain revealed that the protein can bind to lysine-acetylated peptides derived from major acetylation sites on histones H3 or H4 in a highly specific manner, and the interaction is dependent upon acetylation of lysine [8]. This observation was confirmed subsequently by the NMR study of the human GCN5 bromodomain binding to lysine-acety-

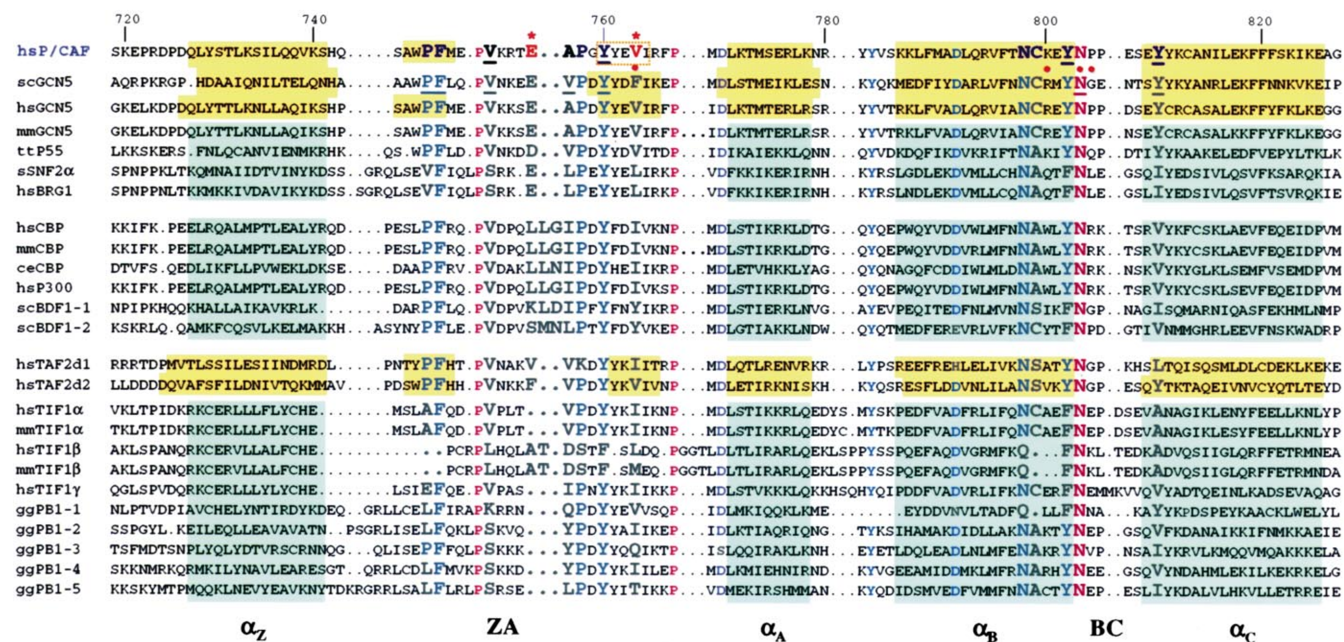

Fig. 2. Structure-based sequence alignment of a selected number of bromodomains. The sequences were aligned based on the experimentally determined structures of five bromodomains, highlighted in orange. Note that in the P/CAF bromodomain a short helix in the ZA loop comprising the YYEVI sequence (boxed by dashed lines) was only observed in the free but not in the peptide-bound form. The predicted secondary structures in the other bromodomains are shaded in green. Bromodomains are grouped on the basis of the predicted structural similarities. Residue numbers of the P/CAF bromodomain are indicated above its sequence. Absolutely (purple) or highly (blue) conserved residues are highlighted. In P/CAF, the residues directly interacting with the Tat peptide are shown in a larger font size, and residues essential for the acetyl-lysine binding, as supported by mutagenesis, are underlined. P/CAF residues important for ligand specificity via interactions with the residues flanking AcK in the Tat peptide are highlighted by red asterisks. In scGCN5, protein residues contacting the acetyl-lysine in the H4 peptide are underlined, and residues contacting other parts of the peptide are indicated by red dots.

lated histone H4 peptides [11]. The NMR structural analysis of the P/CAF bromodomain in complex with acetyl-histamine, a chemical analog of acetyl-lysine, showed that the acetyl-lysine binding site is indeed localized to the hydrophobic cavity between the ZA and BC loops [8]. The intermolecular interactions are largely hydrophobic in nature, with the methyl and methylene groups of acetyl-histamine making extensive contacts with the side chains of V752, A757, Y760, Y802, N803 and Y809, which are highly conserved throughout the large family of bromodomains (Fig. 2) [8].

A crystal structure of scGCN5p bromodomain solved in complex with an acetylated peptide derived from histone H4 at K16 (A-AcK-RHRKILRNSIQGI, where AcK is an N^{ε}-acetyl lysine) reveals the atomic details of the acetyl-lysine recognition (Fig. 1B) [10]. In addition to binding to the conserved hydrophobic and aromatic residues seen in the P/CAF bromodomain, the acetyl-lysine forms a specific hydrogen bond between the oxygen of the acetyl carbonyl group and the side chain amide nitrogen of an absolutely conserved asparagine, N407 (corresponding to N803 in P/CAF) (Fig. 1C). A network of water-mediated hydrogen bonds with protein backbone carbonyl groups at the base of the cleft also contributes to acetyl-lysine binding. Site-directed mutagenesis confirmed the critical role of these amino acid residues in binding to acetyl-lysine, suggesting that acetyl-lysine recognition is a general feature of bromodomains [8]. While the major binding determinant in the scGCN5p bromodomain–H4 peptide complex is the acetylated lysine itself, the protein also has a limited number of contacts with residues at (AcK+2) and (AcK+3) in the H4 peptide. Specifically, binding of the aromatic ring of histidine at (AcK+2) interacts directly with aromatic side chains of Y406 and F367, which are conserved in the bromodomain family (Fig. 1C).

4. Differences of ligand selectivity

The understanding of ligand selectivity of bromodomains is greatly enhanced by the recent structural analysis of a highly selective association between the P/CAF bromodomain and a lysine-acetylated *trans*-activator protein Tat of human immunodeficiency virus type 1 (HIV-1) [26]. Tat stimulates transcriptional activation of the integrated HIV-1 genome and promotes viral replication in infected cells [27–31]. Tat transactivation activity is dependent on acetylation at K50 by the nuclear histone acetyltransferase p300/CBP and consequent association with P/CAF via a bromodomain interaction [32–34]. This bromodomain-mediated interaction results in the release of lysine-acetylated Tat from TAR RNA association, leading to Tat-mediated HIV-1 transcriptional activation [35–37]. Deletion of P/CAF C-terminal region comprising the bromodomain potently abrogated Tat transactivation of integrated, but not unintegrated HIV-1 LTR [35].

The new NMR structure of the P/CAF bromodomain in complex with an acetylated Tat K50 peptide (SYGR-AcK-KRRQR) showed that in addition to the acetyl-lysine, residues flanking both sides of the acetyl-lysine are important for the complex formation (Fig. 1D). Y(AcK−3) forms extensive contacts with Y802 and V763, whereas R(AcK+3) and Q(AcK+4) directly interact with E756. These specific interactions, confirmed by site-directed mutagenesis, confer a highly selective association between the P/CAF bromodomain and Tat [26]. The extensive ligand interactions explain why the

P/CAF bromodomain binds to the Tat AcK50 peptide with a binding affinity ($K_D \sim 10$ μM) about 30-fold higher than that for a histone H4 AcK16 peptide ($K_D \sim 300$ μM) [26]. The differences in ligand selectivity further explain the striking differences in both location and orientation of the bound peptides in the P/CAF and scGCN5 bromodomains – the backbones of the Tat and H4 peptides lie in parallel in the two corresponding structures but are oriented nearly opposite to each other (Fig. 1C and D). Despite these differences, it is interesting to note that scGCN5p binding of H4 H(AcK+2) is reminiscent of P/CAF bromodomain recognition of Tat Y(AcK−3) via residues Y802 and V763, which are equivalent to residues Y406 and F367 in scGCN5p. Because of this similar mode of molecular interaction, the two aromatic residues, which are located in very different positions in the Tat and H4 peptides with respect to the acetyl-lysine, are found surprisingly to be in a nearly identical position in the corresponding bromodomain structures (Fig. 1C and D). High conservation of these residues in bromodomains suggests that selection of an aromatic or hydrophobic residue neighboring the acetyl-lysine is possibly a common mechanism in the bromodomain family (Fig. 2).

5. Perspective

Since bromodomain residues important for acetyl-lysine recognition are largely conserved, binding of lysine-acetylated proteins 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 (Fig. 2), which include (i) variations in the ZA loops, which have relatively low sequence conservation and amino acid deletion or insertion in different bromodomains; and (ii) differences in bromodomain residues that directly interact with residues surrounding acetyl-lysine in a target protein. For instance, E756 in the bromodomain of P/CAF is unique and only present in a small subset of bromodomains from proteins including GCN5. An analogous residue in the bromodomain of CBP or p300 is a leucine residue followed by a two amino acid insertion that is also present in a small subfamily of bromodomains (Fig. 2). Moreover, a short helix corresponding to the AWPFM sequence in the ZA loop of P/CAF (residues 745–749) is likely to be completely missing in the TIF1β bromodomain due to an amino acid deletion. E756 of P/CAF is substituted with a two-residue AT motif in the TIF1β sequence. These differences in the ZA loop may explain why the CBP and TIF1β bromodomains did not interact with Tat AcK50 peptide [26].

In summary, the recent structural studies of bromodomains demonstrate that like *Src* homology 2 (SH2) and phosphotyrosine binding (PTB) domains [38–41], which specifically recognize tyrosine-phosphorylated proteins in signal transduction [42,43], bromodomains can also bind with high selectivity to lysine-acetylated proteins through specific interactions with amino acid residues flanking the acetyl-lysine [10,26]. This new knowledge of ligand specificity will undoubtedly facilitate our understanding of molecular mechanisms underlying specific biological functions of bromodomains in cellular processes including chromatin remodeling and transcriptional activation. These structural studies further emphasize the concept that functional diversity of a conserved protein modular structure is achieved by evolutionary changes of amino acid sequences in the ligand binding site. Tracking such an evolutionary trail on the functional divergence of bromodomains (particularly involving the ZA loop) will require detailed knowledge of protein structure–function relationships, which can be only acquired from three-dimensional structures of new bromodomain/biological ligand complexes.

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