

ACETYLLYSINE-BINDING AND FUNCTION OF BROMODOMAIN-CONTAINING PROTEINS IN CHROMATIN

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1. ABSTRACT

Acetylated histones are generally associated with active chromatin. The bromodomain has recently been identified as a protein module capable of binding to acetylated lysine residues, and hence is able to mediate the recruitment of factors to acetylated chromatin. Functional studies of bromodomain-containing proteins indicate how this domain contributes to the activity of a number of nuclear factors including histone acetyltransferases and chromatin remodelling complexes. Here, we review the characteristics of acetyllysine-binding by bromodomains, discuss associated domains found in these proteins, and address the function of the bromodomain in the context of chromatin. Finally, the modulation of bromodomain binding by neighbouring post-translational modifications within histone tails might provide a mechanism through which combinations of covalent marks could exert control on chromatin function.

2. INTRODUCTION

Post-translational modification of N-terminal tails of histones in eukaryotic chromatin is widely recognised as an important mechanism of genetic regulation. These modifications include acetylation, phosphorylation, methylation, ADP-ribosylation and ubiquitination (1). Acetylation of side chains of lysine residues in the N-termini of all four core histones, first observed by Vincent Allfrey and colleagues (2), is a reversible process whose steady state equilibrium is maintained by the opposing action of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylated chromatin has been observed to correlate with active regions of the genome, and the discovery that many transcriptional co-activators

such as Gcn5p (3), p300/CBP (cAMP response element-binding protein) (4, 5), ATF-2 (activating transcription factor-2) (6), ACTR (7) and SRC-1 (steroid receptor coactivator-1) (8), possess histone acetyltransferase activity further implicates histone acetylation in transcriptional activation (reviewed in 9, 10, 11, 12, 13, 14, 15).

Histone acetylation has been shown to alter the physical properties of chromatin and may help overcome restrictive chromatin structure to allow gene transcription (11, 16). However, it is becoming increasingly evident that histone tails which are acetylated or subject to other modifications may act as binding sites for effectors which could mediate further alterations in the status of associated chromatin (15, 17, 18). This hypothesis is particularly attractive as it provides a nucleosome-mediated regulatory mechanism with great capacity for complexity. In this connection, a protein module known as the bromodomain, widely distributed amongst chromatin-associated factors, is of particular interest since it was shown to bind specifically to acetylated lysine residues, and can therefore mediate the association between bromodomain-containing proteins and acetylated nucleosomes in active chromatin.

Here, we review the major functional classes of bromodomain-containing proteins found in chromatin, discussing their structure and associated domains commonly found in these proteins. Binding analyses and structural studies that reveal the molecular basis of bromodomain interaction with acetyllysine residues are discussed. Evidence as to how the bromodomain-acetyllysine interaction might contribute to a variety of functions within chromatin is considered. Finally, the effect

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of multiple bromodomains within some proteins and multiply modified histone tails upon these interactions is addressed.

3. BROMODOMAIN DISCOVERY AND NOMENCLATURE

The bromodomain (reviewed in 19) is a structural domain of 110 amino acids, containing a central motif sequence of 60 amino acids, which is conserved from yeast through to mammals (Table 1), and has been identified in over 40 proteins. It was first identified as a conserved domain in *Drosophila* brahma (from which it derives its name) and female-sterile homeotic proteins, the yeast Swi2p/Snf2p and the human TAF_{II}250 protein (40). Virtually all of these proteins are known to function in the nucleus and are implicated in controlling chromatin remodelling and gene transcription. The ability of bromodomains in these proteins to bind acetylated nucleosomes may allow the intimate contact between these proteins and active chromatin that is required for their function.

4. ACETYLLYSINE BINDING BY BROMODOMAINS

A number of studies have shown the interaction between bromodomains and peptides derived from histone N-terminal tails, in a manner dependent on acetylation of lysine residues within the peptide. NMR experiments indicate that the P/CAF (p300/CBP-associated factor) bromodomain interacts specifically with a histone H4 N-terminal peptide acetylated at Lys8, and a histone H3 peptide acetylated at Lys14, which are major acetylation sites *in vivo* (79). Unacetylated versions of these peptides did not interact in the same assay. Similarly, the histone H4 tail was shown to interact with the double bromodomain of TAF_{II}250 by isothermal titration calorimetry in an acetylation-dependent manner (80). Binding of the histone H4 tail by the Gcn5p bromodomain is also acetylation-dependent (81). Although interaction between unmodified histone H3 and H4 peptides and the Gcn5p bromodomain have been reported (82), it now seems likely that this results from a secondary interaction between the bromodomain and amino acids adjacent to the acetylated lysine, which enhances the specificity of acetylation-dependent binding (81; discussed further below).

High-resolution structural studies on bromodomains from P/CAF, TAF_{II}250, and Gcn5p indicate a common fold and mode of ligand binding (79, 80, 82, 83). The domain forms a four-helical bundle with unusual left-handed topology. The acetyllysine side-chain binds within a deep cleft formed from the ZA and BC loops of the domain, with extensive packing interactions with the aliphatic part of the side-chain. The importance of these hydrophobic interactions for binding to acetyllysine is shown by the reduction in binding affinity of mutant P/CAF bromodomains lacking hydrophobic side-chains that line this cleft (79). The carbonyl group of the acetylated residue is oriented through a hydrogen bond with an Asn residue of the bromodomain. Specificity for acetylated lysine is

ensured by the unfavourability of binding of the positively charged unmodified lysine in this hydrophobic cavity, consistent with binding study data. The structure of the Gcn5p bromodomain with a histone H4 peptide ligand acetylated at Lys16 reveals a secondary binding interaction involving residues at the K+2 and K+3 positions, where K is the position of the acetylated lysine. His18 and Arg19 make specific interactions with the surface of the bromodomain (81). Such an arrangement is highly reminiscent of the interaction between phosphotyrosine-containing peptides and SH2 domains, in which residues C-terminal to the phosphorylation site contribute to the specificity of binding. This manner of binding allows recognition to be both specific and yet strictly dependent on the covalent modification of a particular lysine. Therefore, it is probable that bromodomain binding will be specific for particular lysines, allowing acetylation of different sites to bring about particular functions.

5. OTHER SEQUENCE MOTIFS IN BROMODOMAIN-CONTAINING PROTEINS

Bromodomains are found in a wide variety of proteins that have some functional role in transcriptional activation. These include chromatin-remodelling proteins, transcriptional co-activators, transcription factors and some nuclear HATs (Table 1). They have been found to occur as often as five times in one protein (36) but typically appear only once or twice, and can be positioned at the N- or C-termini or within a polypeptide sequence (reviewed in 19).

Proteins that contain the bromodomain also possess additional protein motifs (Table 1). The PHD (plant homeodomain) finger is ~60 amino acid domain that possesses a zinc finger motif thought to be involved in protein-protein interactions and is conserved, along with the bromodomain, in the TIF1 (transcription intermediary factor-1) family of proteins (54, 57–59), KAP-1 (48), BPTF (bromodomain PHD finger transcription factor) (63), the BAZ (bromodomain adjacent zinc finger) family of proteins (39) and WCRF180 (Williams syndrome transcription factor-related chromatin remodelling factor) (44). The conservation of the PHD finger and bromodomain suggests that the two domains may biochemically function together. In support of this hypothesis, it has been recently shown that the optimal transcriptional repression of KAP-1 is dependent on the presence of both the PHD domain and bromodomain (48; see below). In addition, Rsc1p and Rsc2p possess a BAH (bromo-adjacent homology) domain and AT hook along with their two bromodomains (34). The BAH domain is of unknown function and was first identified in chicken polybromo (36) and the AT hook is a short DNA-binding motif first identified in the high mobility group HMG I(Y) (84). One of the bromodomains, the BAH domain and the AT hook have been found essential for Rsc1p and Rsc2p function, although they are not required for assembly of these proteins into the RSC (remodels the structure of chromatin) complex itself (34; discussed further below).

Bromodomain-containing proteins in chromatin

Table 1. Bromodomain-containing proteins

PROTEIN	ORGANISM	BROMODOMAINS	OTHER DOMAINS	FUNCTION	REF
HISTONE ACETYLTRANSFERASES					
Gcn5p	Yeast	1	Acetyl-CoA binding domain	Transcriptional co-activator and histone acetyltransferase	20
Gcn5p	Human	1	Acetyl-CoA binding domain	Transcriptional co-activator and histone acetyltransferase	21
TAF _{II} 230	<i>Drosophila</i>	2	AT hook	TFIID 230 kDa subunit and histone acetyltransferase	22
TAF _{II} 250	Hamster	2	AT hook and kinase domain	TFIID 250 kDa subunit and histone acetyltransferase	23
CBP	Mouse	1	ZZ domain, TAZ domain, PHD domain, acetyl-CoA binding domain	Transcriptional co-activator and histone acetyltransferase	25, 26
CBP	Human	1	ZZ domain, TAZ domain, PHD domain, acetyl-CoA binding domain	Transcriptional co-activator and histone acetyltransferase	25, 26
CBP1 (YNJ1)	<i>C. elegans</i>	1		CBP homologue	27
p300	Human	1	ZZ domain, TAZ domain, PHD domain, acetyl CoA binding domain	Transcriptional co-activator and histone acetyltransferase	28
P/CAF	Human	1	Acetyl-CoA binding domain	Histone acetyltransferase	21
p55	<i>T. thermophila</i>			Histone acetyltransferase	29
CHROMATIN REMODELLING PROTEINS					
SNF2 α (hBrm)	Human	1	Helicase and ATP binding domain	Brm homologue. Transcriptional co-activator for nuclear receptors	30
SNF2- β (hBrg1)	Human	1	Helicase and ATP binding domain	Transcriptional co-activator for nuclear receptors. Component of human Swi/Snf chromatin remodelling complex	31
SNF2/SWI2	Yeast	1	Helicase and ATP binding domain	Component of the Swi/Snf chromatin remodelling complex	32
Brg1	Mouse	1	Helicase and ATP binding domain	Homologue of <i>Drosophila</i> brahma	33
Rsc1p	Yeast	2	BAH domain and AT hook	Component of the RSC chromatin remodelling complex	34
Rsc2p	Yeast	2	BAH domain and AT hook	Component of the RSC chromatin remodelling complex	34
Rsc4p (YKY8)	Yeast	2		Component of the RSC chromatin remodelling complex	35
polybromo	Chicken	5	AT hook, 2 BAH domain and DNA methyltransferase-like domain	Homology with yeast Rsc1 and Rsc2 and U19102. Maybe present in Swi/Snf	36
U19102	Yeast	2	BAH domain and DNA methyltransferase domain.	Homology with chicken polybromo. Unknown function	37
Acf1	<i>Drosophila</i>	1	PHD domain, 2 novel conserved regions also found in WSTF (named WAC and WAKZ)	Component of the ACF remodelling complex (along with ISWI)	38
BAZ 1A, 1B, 2A and 2B	Human	1	PHD finger	Homology to <i>Drosophila</i> Acf1. Interacts with human homologues of ISWI.	39
BRM (brahma)	<i>Drosophila</i>	1	Helicase domain and ATP binding domain	Homologue of yeast Swi2/Snf2. Transcriptional activator of homeotic genes	40
Brm (cBrm)	Chicken	1	Helicase and ATP binding domain	Homologue of human Brm. Component of Swi/Snf chromatin remodelling complex. Binds to SNF11 (also part of Swi/Snf complex) and Rb.	41
Brg1 (cBrg1)	Chicken	1	Helicase and ATP binding domain	Homologue of human Brg1. Component of Swi/Snf chromatin remodelling complex. Binds to SNF11 (also part of Swi/Snf complex) and Rb.	41
Sth1p	Yeast	1	Helicase domain and ATP binding site	ATPase present in RSC	42, 43
WCRF180	Human	1	PHD finger	Homology to WSTF and to <i>Drosophila</i> ISWI. Transcriptional regulator and component of WCRF, a chromatin remodelling complex from HeLa cells	44
TRANSCRIPTIONAL CO-ACTIVATORS					
FSH	<i>Drosophila</i>	2	Kinase domain and ET domain	Interacts with trithorax transcription factor and required maternally for proper expression of homeotic genes.	40, 45
RING3 (Brd2)	Human	2	Kinase domain and ET domain	Homologue of <i>Drosophila</i> FSH. Serine threonine kinase	46, 47
BRDT	Human	2	PEST sequence (characteristic of proteins that undergo rapid degradation)	Homologue to human RING3 and <i>Drosophila</i> FSH.	49
ORFX	Human	2		Homologue of human RING3	50
BR140	Human	1	Zn finger and PHD domain	Homology with TAF _{II} 250. Possible transcriptional co-activator	51
(peregryn)					
BP75	Mouse	1	Potential phosphorylation sites	Homology with human BR140. Binds to the PDZ domain in protein tyrosine phosphatase.	52
Spt7p	Yeast	1		Transcriptional co-activator of TY elements. Component of the SAGA complex	32
TIF1- α	Human	1	RING finger, PHD domain, B box and coiled coil domain	Interacts selectively with the activation domain of estrogen receptor. Interacts with the KRAB silencing domain of KOX1 and with HPI	53

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TIF1- α	Mouse	1	RING finger, PHD domain, B box and coiled coil domain	Interacts with the activation domain of estrogen receptor	54
Lin49	C. elegans	1	PHD domain	Homology with human BR140. Required for the normal development of the mating structures of the adult male tail	55
Fsrg1 (RING3)	Mouse	2	ET motif	Homologue of <i>Drosophila</i> FSH	56
TIF1- γ	Human	1	RING finger, B box, coiled coil domain, and PHD domain	Novel member of the TIF1 gene family but unlike α and β does not interact with nuclear receptors, KOX1 or HP1	57
TIF1- β (KAP1)	Human	1	RING finger, B box, coiled coil domain and PHD domain	TIF-1 gene family member but does not interact with nuclear receptors but with KOX1 and HP1	48, 58
TIF1- β (KRIP1)	Mouse	1	RING finger, coiled coil domain and B box	Interacts with nuclear receptors	59
Rfg7	Human	1	RING finger, coiled coil domain and PHD domain	Homology with TIF1. Possible transcriptional coactivator for nuclear receptors	60
p120	Human	1		Homology with human SMAP. Possible nuclear receptor co-activator	61
Brd1	Yeast	2		Possible transcriptional co-activator	62
OTHER TRANSCRIPTION FACTORS					
BPTF	Human	1	PHD finger, glutamic rich acidic domain, LXXLL motif (possible interaction with nuclear receptors)	Involved in hormonally regulated chromatin mediated transcriptional regulation	63
WSTF	Human	1	PHD finger	Deleted in Williams syndrome and a possible transcription factor	64
Bdf1p	Yeast	2	ET domain and kinase domain	Homologue of yeast BDF2. Transcription factor involved in the transcription of a broad class of genes. Interacts with TAF67 in TFIID	65, 66
Bdf2p	Yeast	2		Homologue of yeast BDF1. Interacts with TAF67 in TFIID.	66
SMAP	Human	1		Putative transcription factor	67
ASH1	Human	1	SET domain, PHD finger and AT hooks	Homology to <i>Drosophila</i> Trithorax protein and resembles ALL1. Possible transcription factor	68
HRX (ALL1)	Human	1	SET domain, PHD finger, CXXC domain and AT hooks	Homology to <i>Drosophila</i> Trithorax protein. Possible transcription factor	69, 70
HRX (ALL1)	Mouse	1	SET domain, PHD finger, CXXC domain and AT hooks	Homology to <i>Drosophila</i> Trithorax protein. Possible transcription factor	71
ADDITIONAL PROTEINS					
SP140 (LY100)	Human	1	PHD finger and SAND domain	Component of the nuclear body	72
MCAP	mouse	2	ET domain	Localises to condensed chromosomes during mitosis following histone H3 phosphorylation	73
BS69	Human	1	PHD finger and MYND domain	Interacts with E1A and c-Myb to inhibit the transcriptional activity of c-Myb	74
TBP7	C. elegans	1	AAA domain and potential ATP binding site	Belongs to the AAA family of ATPases	75
Tbp7p	Yeast	1	AAA domain and potential ATP binding site	Belongs to the AAA family of ATPases	76
Celtix-1	Human	1		Interacts with IRF-2. Co-localises with acetylated H3 and H4 by FISH	77
Brf3	Human	1	PHD finger	Unknown function	78

The chromodomain has recently been demonstrated to function in the recognition of methylated histone H3 lysine 9 that is associated with heterochromatin (85, 86). There are a number of common themes between bromodomain acetyllysine binding and chromodomain methyllysine recognition, including the capacity of these proteins to recruit in other factors to the modified sites. Both domains can also be associated with the enzymatic activities that generate their cognate binding sites, since nuclear HATs nearly always contain bromodomains and the methyllysine-binding, *chromodomain*-containing protein HP1 (heterochromatin protein 1) interacts with a histone methyltransferase, suggesting a mechanism of the spreading of these modifications through chromatin.

6. BIOLOGICAL SIGNIFICANCE AND FUNCTION

Although the structure and acetyllysine-binding of bromodomains is now well established, the precise significance of this interaction to the function of bromodomain-containing proteins remains unclear. In this

section, we address the extent to which acetyllysine-binding activity of bromodomains has been shown to be functionally significant by *in vivo* studies, and how this activity contributes to gene regulation.

6.1. Bromodomain function in histone acetyltransferases

The fact that bromodomains are often present in nuclear HATs suggests that bromodomains may play a key role in the function of these enzymes, which is borne out by biological studies. In yeast, truncated Gcn5p lacking the bromodomain cannot fully complement the phenotype of a *gcn5⁻* strain, and strains lacking the bromodomain show transcription-related defects (20). However, the *in vitro* HAT activity of Gcn5p does not require the bromodomain, nor is the bromodomain required for transcriptional activation by strong activators, although weak activators are reliant on the bromodomain (87). These data suggest that bromodomains control not HAT enzymatic activity itself, but the targeting of this activity, consistent with its molecular function as an interaction motif. This idea is reinforced by studies of Gcn5p when present in the SAGA

(Spt-Ada-Gcn5) complex, one of the large complexes required for the acetylation of nucleosomal substrates (88). The SAGA complex containing Gcn5p lacking its bromodomain is less effective at acetylating nucleosomes than complexes with wild-type Gcn5p (89). *In vivo*, deletion of the bromodomain of Gcn5p alters transcriptional control and attenuates transcriptional activation at the *HIS3* locus in a manner similar to loss of Gcn5p activity (89). These data argue for the involvement of the bromodomain of Gcn5p in recruitment to nucleosomes *in vivo*. However, loss of the bromodomain from Spt7p, another component of the SAGA complex does not result in any obvious phenotypic defects (89, 90).

Note that there is an apparent contradiction in these considerations: the enzyme that deposits acetyl groups on lysine residues contains bromodomains and requires acetyllysines for its interactions and function. By analogy with lysine methylation and chromodomains, it may be that this allows spread of histone acetylation, possibly to adjacent nucleosomes or to other lysines on the same nucleosome. Alternatively, as with DNA methylation, this may provide a mechanism for transmitting the modified state whereby existing acetylated nucleosomes with these enzymes bound produce the modification of newly deposited nucleosomes during DNA replication (18, 91). These issues remain to be resolved.

6.2. Bromodomain function in remodelling complexes

Bromodomains are also present in many chromatin-remodelling complexes (Table 1). Loss of the bromodomain of the yeast Swi2p/Snf2p component of the SWI/SNF remodelling complex causes no discernible phenotype (32), and similar results are seen in its *Drosophila* homologue, *brahma* (*brm*) (92). The bromodomain of the human homologue, *hbrm*, is not needed for *hbrm*-mediated transcriptional activation by the glucocorticoid receptor, but its deletion does lead to loss of *hbrm* nuclear localisation and decreases the stability of the protein (30). The yeast RSC remodelling complex may have a more widespread role in genome-wide regulation than SWI/SNF (93). RSC exists in at least two forms, which contain either the Rsc1p or Rsc2p gene product (34). These two proteins are homologous and contain two bromodomains at their N-termini (34). Study of deletion mutants under a variety of growth conditions indicates that the second bromodomain of both Rsc1p and Rsc2p is absolutely required for growth and strains harbouring a specific deletion of this domain in either Rsc1p or Rsc2p have identical phenotypes to *rsc* null mutants (34). In addition, the first bromodomain of Rsc2p is needed for growth only under certain conditions (34). These data not only provide genetic evidence for an absolute requirement for bromodomain function within a remodelling complex but also suggest that bromodomains within the RSC confer distinct properties on the complex. This could be a result of differing binding specificities of the two bromodomains within Rsc1p or Rsc2p.

6.3. Bromodomains and orchestration of chromatin remodelling

There is increasing evidence for a central role for bromodomains in the functional interplay between histone acetylation and ATP-dependent remodelling which is

important for many genes (reviewed in 94). A number of recent studies have shown that recruitment of HATs and ATP-dependent remodellers occurs in an ordered fashion (95, 96, 97, 98). For example, efficient recruitment of hSWI/SNF to the IFN- β (interferon- β) promoter is dependent on prior acetylation by hGcn5p (95). The latter phenomenon could be explained by the anchoring of hSWI/SNF to acetylated histone tails by the bromodomain of the BRG1 subunit of hSWI/SNF. In contrast to the order of events at the IFN- β promoter, SWI/SNF is recruited to the yeast *HO* promoter initially through interactions with the DNA-binding activator Swi5p, and this complex then recruits the SAGA acetylase complex (96). Interestingly however, SWI/SNF persists at the *HO* promoter long after Swi5p, which associates only transiently, has departed. Recent data indicate that histone acetylation stabilises the association of SWI/SNF with the *HO* promoter in an *in vitro* system (99), which is again suggestive of a role for bromodomains in the fixing of remodellers at acetylated chromatin.

The importance of bromodomains in the functional interplay between histone acetylation and chromatin remodelling is also demonstrated in a recent study on a promoter-reporter gene construct in yeast (100). This construct could be activated in response to amino-acid starvation by the DNA-binding protein Gcn4p, in a manner that required both catalytically active Gcn5p and the Swi2p subunit of the SWI/SNF chromatin-remodelling complex. Activation of transcription correlated with nucleosome remodelling, and remodelling could not occur in the absence of Gcn5p-mediated histone acetylation. Chromatin immunoprecipitation (ChIP) assays using deletion mutants indicated that histone acetylation occurred independently of remodelling. Taken together, the data presented suggest there might be a functional link between histone acetylation and chromatin remodelling. Strains in which the bromodomain of Gcn5p was either deleted or had critical residues for acetyl-lysine recognition mutated could not support chromatin remodelling, despite the fact that histone acetylation was actually increased. A surprising and important observation was that recruitment of SWI/SNF to the promoter was diminished in Gcn5p bromodomain mutants, despite the fact that SWI/SNF can associate with the promoter efficiently in the absence of Gcn5p altogether, via direct interaction with Gcn4p. Thus a stable association of SWI/SNF with the acetylated promoter requires a functional bromodomain to be present in Gcn5p. There are a number of possible interpretations for this result. The authors suggest that histone acetylation could disrupt somehow the interaction of SWI/SNF with the promoter, and that the bromodomain can reverse this effect. One possibility is that the bromodomain, stabilised on the acetylated chromatin, can act as an interaction surface for the SWI/SNF complex in the context of the hyperacetylated promoter. Another explanation for these data is that the bromodomain of Gcn5p may function to anchor the HAT to chromatin independent of its interaction with the activator (94). This might allow release of the activator-binding site by

Gcn5p, leaving the activator free to interact with SWI/SNF.

6.4. Other functions for bromodomains

Other evidence presenting the bromodomain as a key interaction surface at promoters comes from the study of the KAP-1 co-repressor, a protein that helps to establish transcriptional repression when targeted by DNA-binding transcription factors containing a Kruppel-associated box (KRAB) domain. KAP-1 and other members of this transcription factor family have a bromodomain located at their C-terminus, preceded by a PHD finger domain, another domain frequently found in proteins that interact with chromatin. Schultz *et al* (2001) present evidence that the PHD finger and bromodomain of KAP-1 form an interdependent structure that is capable of repressing transcription when tethered to DNA (48). Mutational analysis shows that alterations in either domain can abrogate repression, although domain-swap chimeric proteins implicate the bromodomain as the major interactive surface. Two-hybrid screens identified the Mi-2 α protein as an interaction partner for the KAP-1 PHD/bromodomain unit, verified *in vivo* by co-immunoprecipitation. Mi-2 α exists as a component of a multiprotein complex that contains the histone deacetylase HDAC1. The importance of the recruitment of HDAC activity in KRAB-mediated repression is highlighted by the fact that treatment of cells with the HDAC inhibitor trichostatin A (TSA) can partially reverse repression by the PHD/bromodomain unit of KAP-1. In KAP-1-mediated repression it therefore appears that the bromodomain, in co-operation with the PHD finger, forms a surface for interaction with other chromatin-modifying complexes (in this case a Mi-2 α - and HDAC1-containing multiprotein complex). A central role for the recognition of acetyl-lysine residues by the KAP-1 bromodomain is expected given the sequence conservation of the bromodomain with those known to function in acetyl-lysine recognition, and the fact that mutations that specifically compromise the acetyl-lysine binding site prevent repression. The state of histone acetylation is yet to be studied for this example, but it would be plausible that acetylated histones at this promoter allow the stabilisation of KAP-1 on chromatin so that other activities such as deacetylases may be recruited. Conserved charged patches, which could form interaction surfaces, distinct from the acetyllysine-binding site are observed in the structure of the TAF_{II}250 double bromodomain (80). It is interesting that KAP-1 can also recruit, through other interactions, HP1, a protein involved in the formation and maintenance of heterochromatin. KAP-1 may therefore function to co-ordinate a number of events in the establishment of repression. Heterochromatin formation involves the methylation of histones by methyltransferases, which can in turn interact with HP1. This would potentially provide a mechanism by which the recognition of one histone modification might bring about changes to the array of modifications present (e.g. deacetylation, methylation) and hence alter status of the chromatin.

Recent data suggest that the bromodomain of CBP can interact with the transcription factor Elk-1 (101).

Induction of the *c-fos* proto-oncogene on serum stimulation is dependent on the arrival of signals at a ternary complex bound to the serum response element of the *c-fos* promoter (102), and involves HAT co-activators such as CBP (103, 104, 105). The rapidity of *c-fos* induction led researchers to test for a pre-induction association of CBP at the promoter. It is shown that the bromodomain of CBP can interact with the Elk-1 component of the ternary complex and that in transient transfection assays the bromodomain alone can function in a dominant negative manner (101). The latter effect is reversed by addition of the other domains of CBP to the bromodomain (101). It is currently unclear whether this effect is acetylation-dependent, but one possibility is that acetylation of Elk-1, for which there is reported to be preliminary evidence ("data not shown" in 101), facilitates this interaction. This signalling-independent interaction could facilitate a later conformational change in the transcription factor complex allowing it to utilise signalling-dependent contacts, which would be permissive for transcription activation.

The bromodomain of human Gcn5p is also reported to interact with the Ku70 subunit of DNA-dependent protein kinase (DNA-PK) (106). This interaction allows the recruitment of the kinase to hGcn5p and the catalytic subunit of DNA-PK can phosphorylate hGcn5p (106). Phosphorylation of hGcn5p inhibits HAT activity both *in vitro* and *in vivo* (106). It is unclear whether the hGcn5p bromodomain-Ku70 interaction is acetylation-dependent.

The bromodomain of p300 is required for efficient acetylation of the transcription factor and proto-oncogene *c-Myb* *in vitro*. *In vitro* this modification enhances DNA-binding activity of *c-Myb* (107).

In overview, bromodomains are clearly important for protein-protein interactions in the transcription-related proteins described in this section, but the extent to which these interactions are dependent on acetylation of some of its partners' remains to be addressed.

7. BROMODOMAIN MUTATIONS IN HUMAN DISEASE

The *in vivo* importance of bromodomain function is underscored by the occurrence of bromodomain-associated mutations in human, tumour-derived cell lines. The HAT p300 is a tumour suppressor gene and inactivation of both alleles of p300 has been observed in a number of tumours. A cervical cancer cell line shows a homozygous, in-frame deletion resulting in specific loss of the bromodomain of this transcription coactivator (108). This cell line is defective in TGF- β - (transforming growth factor- β -) signalling-induced activation of the p21 promoter. TGF- β responsiveness of the reporter gene is regained by reintroduction of the wild-type p300. The bromodomain of p300 is therefore important for the function of this coactivator, which is necessary for the activation of growth-suppressive genes. The fusion of the CBP gene to the mixed-linkage leukaemia (*MLL*) gene as a result of chromosomal translocation is associated with acute leukaemia (109). Joining of the HAT and

bromodomain of CBP to MLL are sufficient for the transforming activity of this gene fusion (110). Whilst the HAT domain alone fused to MLL could cause some increase in proliferation both domains were required for full transforming activity. A possible mechanism for transformation by this fusion is the constitutive acetylation of histones at genes targeted by the MLL protein. The bromodomain would promote or regulate this activity in a manner that enhances growth deregulation.

8. THE SIGNIFICANCE OF MULTIPLE BROMODOMAINS AND MULTIPLE HISTONE TAIL MODIFICATIONS

Histone tails are subject to a diverse range of posttranslational modifications, and it is conceivable these form part of a complex biochemical code, where multiple residues modified in a specific combination might be used to denote specific status for a region of chromatin (15, 17, 18). Multiple modifications of histone tails may influence the interaction of bromodomain-containing proteins with nucleosomes in two opposing ways; (1) enhancement of binding by the presence of multiple bromodomains in a single protein, or (2) prevention of binding of any particular bromodomain to its acetyllysine target by the presence of other occluding modifications at nearby sites.

The occurrence of multiple bromodomains within a single polypeptide is of interest since it is known that single nucleosomes may become multiply acetylated both at particular sites within a single tail, and on more than one of the eight tails which protrude from the nucleosome. The basal transcription factor TAF_{II}250 contains two tandem bromodomains, and studies of this double bromodomain module suggest that two acetylated lysine residues on the same tail can be simultaneously engaged (80). Histone H4 tails which were multiply acetylated, either at the K8/K16 and K5/K12 pairs or at all four of these lysines had significantly higher affinity for the protein module than a singly-acetylated peptide. The K5/K12 diacetylpeptide was most strongly bound. The crystal structure of human TAF_{II}250 indicates each of the two bromodomains assumes the previously observed conformation, with their relative orientations fixed through two clusters of interdomain contacts, placing the two acetyllysine-binding sites approximately 25 angstroms apart. Jacobsen *et al* (1999) argue that a spacing of approximately seven residues between two acetylated lysines would allow both binding sites of the double bromodomain to be simultaneously occupied by residues on the same peptide (80). This spacing is consistent with the six and seven residues between the K8/K16 and K5/K12 pairs respectively, and thus is compatible with the binding data that suggest that two acetyllysines on the H4 peptides can be bound at the same time. However, when Owen *et al* (2001) superimpose their Gcn5p bromodomain/H4 peptide structure on that of the TAF_{II}250 bromodomain, they observe a distance of 29 angstroms between the α -carbons of the bound acetyllysine residues (81). This distance is too far to simultaneously accommodate both of the acetylated residues of the K8/K16 or K5/K12 pairs, and would require at least 10 residues between the two acetyllysines. The generated structure

would allow acetyl-K5 and acetyl-K16 to be simultaneously bound, but it is unclear how this model is reconciled with the binding studies of K8/K16 and K5/K12 acetylated peptides of Jacobsen *et al* (1999) (80). Nonetheless, the recognition of multiply-acetylated nucleosomes by multi-bromodomain proteins is one way in which acetylation at a number of sites could contribute to factor recruitment, and in the case of TAF_{II}250 this would aid the binding of the basal transcription machinery at chromatin regions acetylated in a manner known to denote "active" chromatin. The simultaneous engagement of more than one acetyllysine would enhance the affinity of the interaction, and could also make recruitment dependent on the activity of more than one HAT if the sites were targeted by different acetyltransferases.

Converse to the enhancement of binding described above, it is equally possible that adjacent modifications may actually interfere with the interaction between the bromodomain and its target. For example, the presence of phosphate at serine 10 on histone H3 tails may interfere with the recognition by bromodomains of acetylated lysines at positions 9 and/or 14 on the same tail. Evidence that this type of occlusion of protein-protein interaction does occur comes from studies of site- and modification-specific antibodies used as tools to probe the state of histone tails. Phosphorylation of serine 10 of histone H3 is correlated with the induction of immediate-early genes following application of mitogenic or stressful stimuli (reviewed in 111). Recognition by an antibody raised against histone H3 phosphorylated at serine 10 is specifically ablated by acetylation of lysine 14 (112). Furthermore, some antibodies that recognise acetylated lysines on the H3 tail are also prevented from binding those acetyllysines by the presence of phosphate at serine 10 (Clayton, Rose and Mahadevan, unpublished data). Finally, there is also evidence that one acetyllysine residue may interfere with antibody recognition to a second acetyllysine residue (113, 114). Whilst these are clearly non-physiological recognition processes, it indicates how protein recognition is affected by the modification status of adjacent sites. It is interesting to note that a bromodomain-containing protein known as mitotic chromosome-associated protein (MCAP) is targeted to mitotic chromatin during the G2-M transition, when histone acetylation is generally low, and chromosomal localisation occurs subsequent to the stoichiometric phosphorylation of histone H3 that is vital for proper chromosome condensation and segregation (73). It will be interesting to see whether this targeting is dependent on H3 phosphorylation, and whether the bromodomain plays a role in this process.

In addition to the effects of modification of neighbouring residues, it is notable that histone H3 lysine 9 can be either acetylated or methylated. It is therefore possible that different proteins could be recruited to the same region of the histone tail according to which modification is present at a particular time. It is also interesting that in the case of KAP-1 the recognition of acetyllysines might help to co-ordinate the deacetylation and methylation of these sites by recruiting other histone-modifying activities (48). The capacity exists for a substantial interplay between modifications at distinct sites and different modifications at the same site.

9. CONCLUSIONS

It is clear that the bromodomain is a key protein module which functions in conjunction with histone acetylation, most likely through recruiting or facilitating the function of other effectors upon active chromatin. Definition of the specificity of interactions between particular bromodomains and certain acetylated lysines relevant to particular biochemical processes requires much further work, as does the identity of HATs that act to generate these bromodomain binding sites. The capacity for other modifications to influence bromodomain recognition is another interesting area for future research that will provide insights into how the system functions as a whole. Finally, acetylation is a dynamic modification targeted to many factors other than histones involved in gene expression (reviewed in 115). It will be interesting to see if bromodomain-containing proteins will turn out to be capable of acetylation-dependent binding to non-histone proteins, which would place the bromodomain as a key player in mediating networks of signal-dependent, protein-protein interactions that modulate genetic regulation.

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Abbreviations: ACF, ATP-utilizing chromatin assembly and remodelling factor; BAH, bromodomain adjacent homology; BAZ, bromodomain adjacent zinc finger; BPTF,

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bromodomain PHD finger transcription factor; BRDT, bromodomain testis-specific; CBP, cAMP response element binding protein; FISH, fluorescence *in situ* hybridisation; FSH, female sterile homeotic; HP1, heterochromatin protein 1; IRF-2, interferon-regulatory factor-2; KRAB, Kruppel-associated box; MCAP, mitotic chromosome-associated protein; P/CAF, p300/CBP-associated factor; PHD, plant homeodomain; RSC, remodel the structure of chromatin; SMAP, skeletal muscle abundant protein; TIF, transcription intermediary factor; WCRF, Williams syndrome transcription factor-related chromatin remodelling factor; WSTF, Williams syndrome transcription factor.

Key Words: Bromodomain, Chromatin, Histone Acetyltransferase, Chromatin Remodelling, Histone Modifications, Review

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