Epigenetics

DOI: 10.1002/ange.201002724

Interaction of Propionylated and Butyrylated Histone H3 Lysine Marks with Brd4 Bromodomains**

*Friederike Vollmuth and Matthias Geyer**

The regulation of eukaryotic gene expression is determined by modifications in the chromatin structure. The highly variable pattern of modifications in the histone chains is described as the histone code. Two sets of histone proteins H2A, H2B, H3, and H4 form the octameric core of each nucleosome. Two turns of genomic DNA are wound around this octameric core to form the packaging of chromatin structures. Histone proteins are subject to a wide array of posttranslational modifications, including methylation, citrullination, acetylation, phosphorylation, ubiquitination, and sumoylation.[1,2] These histone marks determine the state of chromatin dynamics and remodeling; the study of their specific imprint, which outlasts even cell division, is known as epigenetics.

The N-acetylation of lysine residues in histones H3 and H4 is mediated by histone acetyltransferases (HATs), which require acetyl-CoA as a coenzyme to catalyze the reaction.[3,4] The selectivity of these enzymes depends on both their substrates and their environment within the cell. Acetylated histone tail sequences are recognized in turn by bromodomains, a conserved domain structure of approximately 110 amino acids.[5] Brd4 belongs to the BET (bromodomain and extraterminal domain) family of dual bromodomains (BD1 and BD2), which are thought to play important roles in the epigenetic memory and control of transcription as well as viral inheritance across cell division.[6] Besides acetylation, it has recently been shown that lysine residues of histones from yeast and mammalian cells are also propionylated and butyrylated (Figure 1a,b).[7–10] Both modifications are catalyzed by HATs, as shown for p300 and CREB-binding protein, Esa1, PCAF, and bacterial GCN5-related N-acetyltransferases.[10–13] Whereas long-chain acylations, such as myristoylation (C14) and palmitoylation (C16), cause proteins to target cellular membranes,[14,15] the function of protein short-chain propionylation (C3) and butyrylation (C4) is not yet clear.[16,9] In this study, we analyzed the interaction of propionyl- and butyryllysine residues on histone chains with Brd4 bromodomains and the structural basis of this interaction. The binding affinities of both short-chain acylations were similar to or weaker than those observed for lysine acetylation. The additional carbon atoms aligned between a highly conserved Pro–Phe motif, which suggests a general mode of bromodomain recognition.

Brd4 BD1 was crystallized and exposed to the octamer peptide H3K23prop or H3K14buty, as described in the Supporting Information. Complex structures were determined by rigid-body refinement with the free bromodomain structure[17] and refined to a resolution of 1.75 and 1.65 Å, respectively, with excellent overall stereochemistry (see Table S1 in the Supporting Information). The two interconnecting loops ZA and BC that combine the four canonical bromodomain α helices (α_Z, α_A, α_B, α_C) compose the recognition site for binding to the modified lysine residues (Figure 1c). Isothermal titration calorimetry (ITC) was used to determine the dissociation constants and the thermodynamic parameters of the interaction (Table 1). In comparison with the acetylated lysine peptides described earlier,[17] the two propionylated sequences H3K14prop and H3K23prop showed an approximately threefold weaker binding affinity for Brd4 BD1 ($K_d = 337$ and 380 μM, respectively), whereas

Table 1: Thermodynamic analysis (by ITC) of Brd4 bromodomain binding to lysine-acylated histone peptides.

Interaction[a]	K_d [μM]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]	Molar ratio[b]
Brd4 BD1[c] with				
H3K14ac[d]	118 ± 28	−0.59 ± 0.022	4.77	1.33
H3K14prop	337 ± 76	−0.64 ± 0.096	4.08	0.92
H3K14buty	large	n.d.	n.d.	n.d.
H3K23prop	380 ± 105	−0.63 ± 0.116	4.02	1.06
H4K5ac[d]	325 ± 72	−1.99 ± 0.067	2.76	2.35
H4K5acK8ac	38 ± 3.7	−10.04 ± 0.17	−3.99	0.90
Brd4 BD2[c] with				
H3K14ac[d]	327 ± 75	−1.68 ± 0.059	3.07	2.31
H3K14prop	213 ± 15	−1.48 ± 0.047	3.52	1.00
H3K14buty	large	n.d.	n.d.	n.d.
H3K23prop	208 ± 16	−0.86 ± 0.031	4.17	0.96
H4K5ac[d]	107 ± 23	−1.81 ± 0.060	3.61	1.91
H4K5acK8ac	212 ± 35	−6.35 ± 0.50	−1.34	1.21

[a] All measurements were made at 25 °C. [b] BD1/peptide or BD2/peptide. [c] Brd4 BD1 comprised residues 42–168; Brd4 BD2 comprised residues 349–464. [d] Measurements were taken from Ref. [17].

[*] F. Vollmuth, Priv.-Doz. Dr. M. Geyer
Abteilung Physikalische Biochemie
Max-Planck-Institut für molekulare Physiologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-2399
E-mail: matthias.geyer@mpi-dortmund.mpg.de

[**] We thank Diana Ludwig for excellent technical assistance, the MPI Dortmund–Heidelberg X-ray community for diffraction-data collection, the staff at PSI Villingen, Switzerland, for access to beamline X10SA, Wulf Blankenfeldt for crystallographic advice, and Christian Herrmann and Mark Wehner for access to a microcalorimeter. F.V. is a fellow of the IMPRS in Chemical Biology, Dortmund. This research was supported by a grant from the Deutsche Forschungsgemeinschaft to M.G. (GE-976/5). Atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank (www.rcsb.org) under PDB accession numbers 3MUK and 3MUL.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201002724.

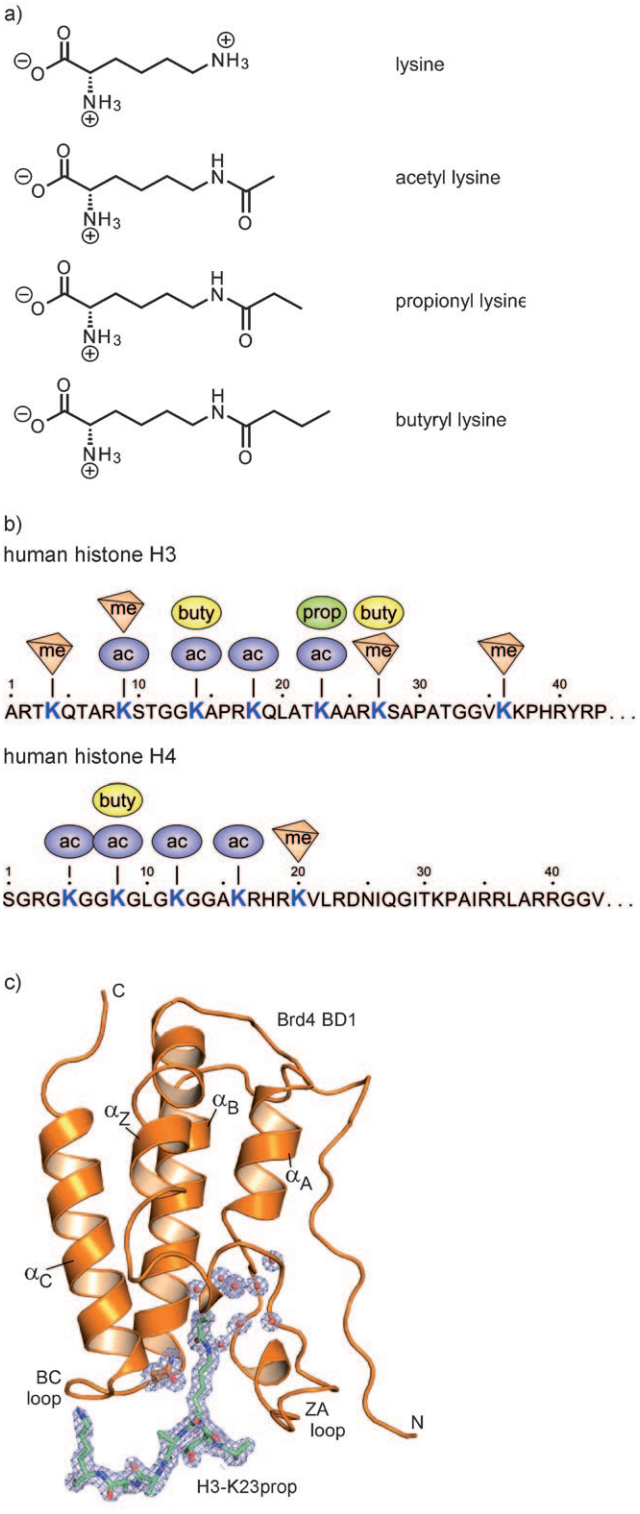

Figure 1. Lysine acyl modifications in histones. a) Nζ-acylation of lysine residues. Acetyllysine is extended to propionyl- and butyryllysine by one and two additional methylene groups, respectively. b) Epigenetic marks on lysine residues in histone H3 and H4 N-terminal sequences. Lysine sites that are known to become methylated (me) or acetylated (ac) are labeled. Recent studies identified additional propionylation (prop) and butyrylation (buty) marks in human and yeast histones.[7,8] c) Structure of the H3K23prop peptide bound to Brd4 BD1. Displayed is the electron-density omit map of the histone peptide at 1.0 σ from diffraction data recorded to a resolution of 1.75 Å.

the affinity of the butyrylated peptide was too weak to be measured. For BD2, propionylated and acetylated peptides showed similar binding affinities (Figure 2a), whereas again the dissociation constant of H3K14buty could not be determined. The binding of Brd4 bromodomains to a dodecamer peptide containing twin histone-acetylation marks, H3K5acK8ac, was also analyzed by ITC. A specific interaction of this motif with BD1 of BrdT, another BET family bromodomain, was recently described.[18] Indeed, a significant increase in binding affinity for Brd4 BD1 was observed (38 μM, Figure 2b) relative to that of the singly acetylated

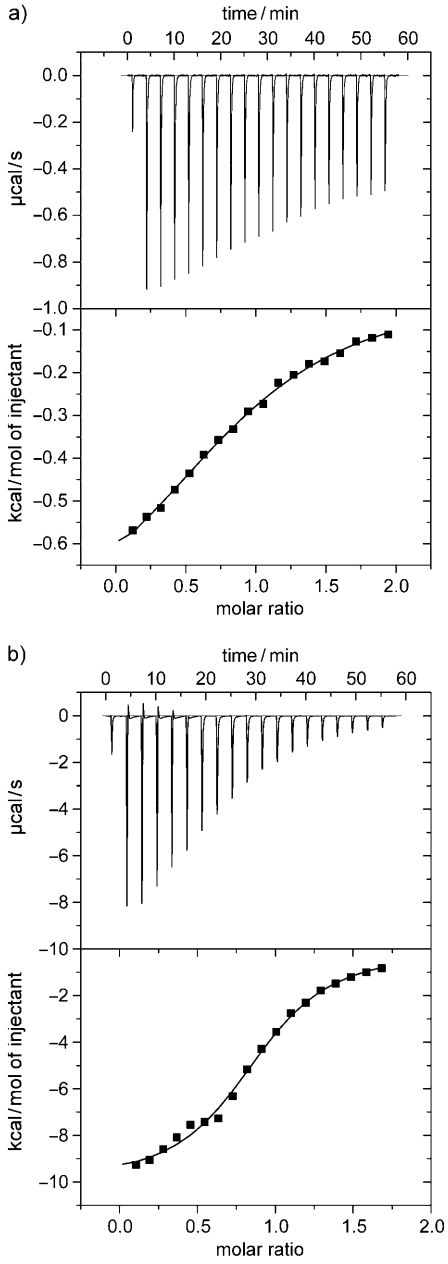

Figure 2. Isothermal titration calorimetry measurement of the interactions between Brd4 bromodomains and histone sequences. a) ITC measurements of H3K23prop peptide binding to Brd4 BD2 revealed a dissociation constant of 208 μM. b) The binding of BD1 to the twin-acetylation motif H4K5acK8ac showed a K_d value of 38 μM.

(K5ac) variant (325 μM). The interaction of this twin-acetylation motif with Brd4 was as tight as reported for BrdT; likewise, the preferred specificity for BD1 over BD2 could be confirmed for Brd4. As similarly found in BrdT, this specificity might be due to an aspartate residue in the BC loop at position 144 of Brd4 BD1. In contrast, BD2 contains a larger histidine residue at this site. The increased affinity of Brd4 for the H4 twin-acetylation motif K5/K8 supports recent observations of the control of inducible gene expression for primary response genes by a Brd4-mediated transition from promoter-proximal pausing to transcriptional elongation.[19]

The crystal structures of both modified lysine peptides bound to Brd4 BD1 showed that a shell of surrounding water molecules remained intact, whereas the additional methylene groups kinked outward (Figure 3a,b; see also Figure S1 in the Supporting Information). The hydrophobic moieties aligned within distances of 3.7–4.0 Å in a hydrophobic groove spanned by residues Pro82, Phe83, Val87, and Ile146 (see Figure S1c,d in the Supporting Information). These residues are highly conserved in BET-family bromodomains; only Ile146 changes (to valine) in BD2s (Figure 4a). Residues that interact with H3K23prop within a shell of 3.8 Å aligned exclusively in the ZA and BC loop. Furthermore, water-mediated contacts to the histone peptide were formed by the backbone carbonyl groups of Pro82, Gln85, Met105, and Met132 and the side chains of Tyr97 and Asn140 (Figure 3c). Interestingly, the directionality of the peptide chain varied for different histone ligands as a result of ionic contacts formed ±3 residues from the modified lysine residue (see Figure S2 in the Supporting Information); a similar observation was reported previously.[20] A similar shell of water molecules lining the surface of the binding pocket was observed for the butyrylated histone peptide (see Figure S3 in the Supporting Information). Overall, this interaction network suggests a general mode of bromodomain binding to short-chain histone-acylation marks.

It is surprising that the propionyl- and butyryllysine moieties do not penetrate deeper into the bromodomain binding site and displace a water molecule from the surrounding water shell but rather loop outward, loosely flanked by hydrophobic contacts (Figure 4b). Trapped water molecules in the active site of a protein–ligand interaction contribute significantly to the energy landscape and specificity of binding.[21] The release of one or two water molecules from the recognition site would be expected to increase the entropy of binding and could lead to the formation of additional hydrophobic contacts, for example, to Met132 of Brd4. Both effects would increase the binding affinity for the bromodomain. Instead, the contribution of the entropy is rather similar for all monoacylated H3 peptides (Table 1), which indicates that the discrete network of noncovalent water bonds remains intact. The change in enthalpy is also very small, in line with the overall low binding affinity and the observation that the bromodomain undergoes no significant conformational changes upon ligand binding. From these results it seems that nature has avoided tight binding for the recognition of acylated-histone marks by bromodomains. The activation pattern for gene expression is based on weak binding affinities; that is, histone activation requires a

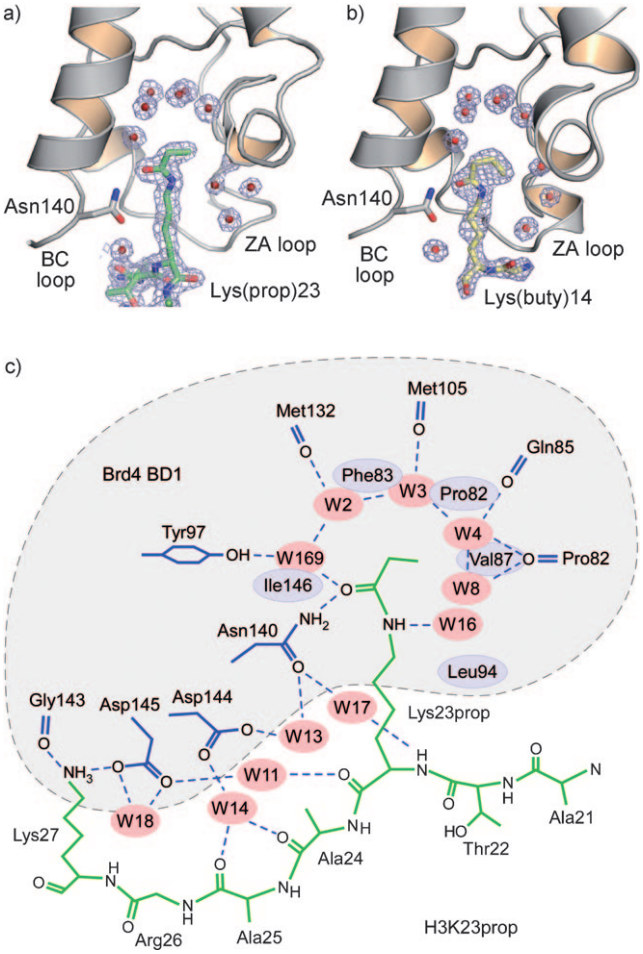

Figure 3. Structural basis of the recognition of propionylated and butyrylated lysine residues by Brd4 bromodomains. a) Electron-density map of H3K23prop bound to Brd4 BD1. Displayed is the omit map at 1.0σ of the modified lysine residue and the surrounding water molecules. b) Electron-density map contoured at 1.0σ of H3K14buty bound to Brd4 BD1 from diffraction data recorded to a resolution of 1.65 Å. c) Interaction map of H3K23prop with Brd4 BD1. Water-mediated contacts are shown by dashed lines. Hydrophobic van der Waals contacts to residues within the binding cavity spanned by Pro82, Phe83, Val87, Leu94, and Ile146 are shown as oval sites. Only atoms visible in the electron-density map of the ligand are displayed.

systemic combination of multiple simultaneous interactions rather than singular high-affinity binding.[22] These multiple modification sites enable high variability based on a small set of modifications, which require cooperative binding and synergistic effects in the recognition of the epigenetic code.

Should propionyl- or butyryllysine in histones now be considered a special epigenetic mark? Short-chain lysine *N*-acyl modifications, such as acetylation (C2), propionylation (C3), and butyrylation (C4), may indeed be regarded as linear analogues to tetrahedral mono-, di-, and trimethylation. However, the observation that the binding affinities for bromodomains remained similar or even decreased relative to that of peptides with acetylated lysine favors a modification that is recognized comparably well. Likewise, there is increasing evidence that the modifying histone acetyltransferases do not differentiate between acetyl-, propionyl-, and

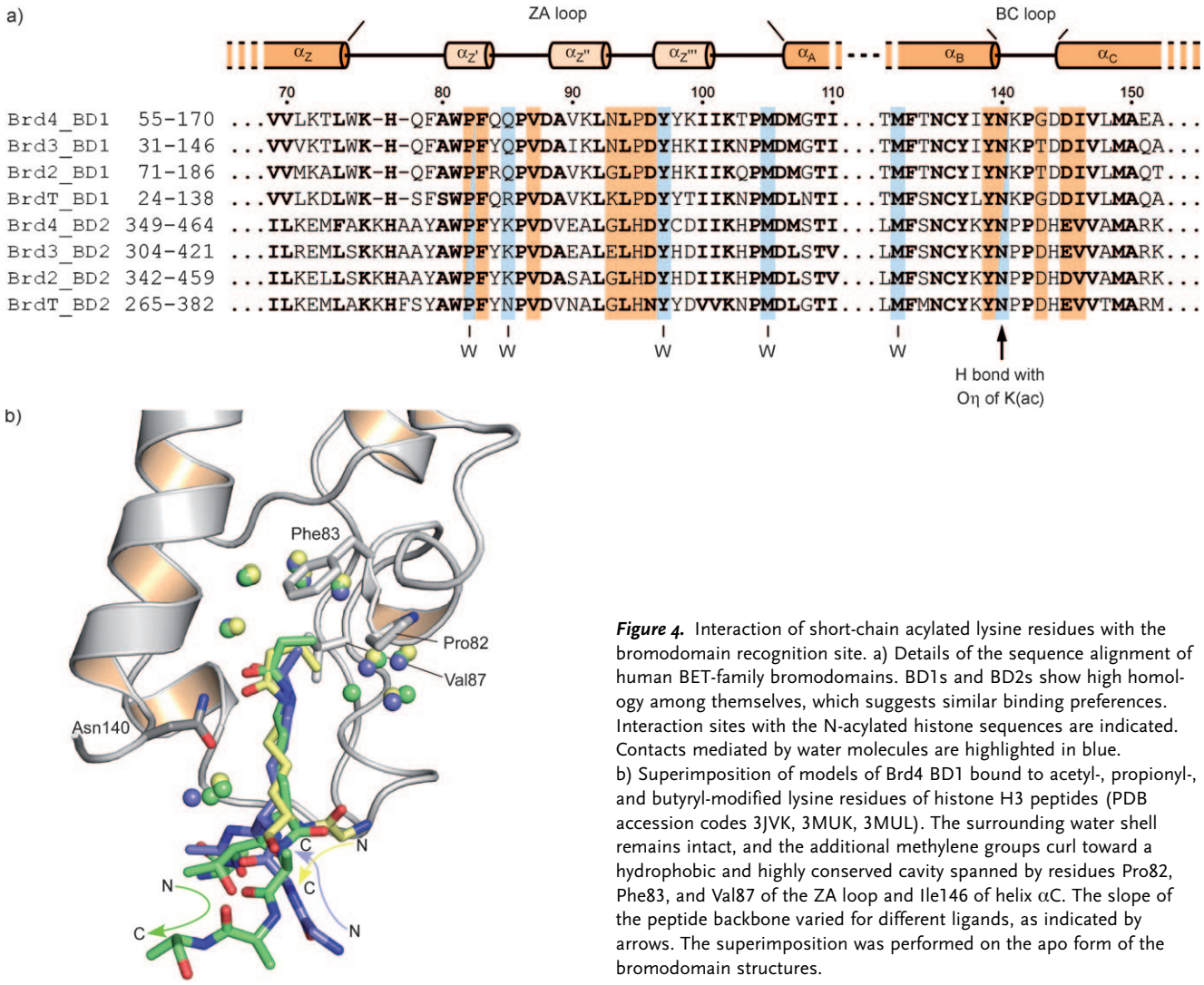

Figure 4. Interaction of short-chain acylated lysine residues with the bromodomain recognition site. a) Details of the sequence alignment of human BET-family bromodomains. BD1s and BD2s show high homology among themselves, which suggests similar binding preferences. Interaction sites with the N-acylated histone sequences are indicated. Contacts mediated by water molecules are highlighted in blue. b) Superimposition of models of Brd4 BD1 bound to acetyl-, propionyl-, and butyryl-modified lysine residues of histone H3 peptides (PDB accession codes 3JVK, 3MUK, 3MUL). The surrounding water shell remains intact, and the additional methylene groups curl toward a hydrophobic and highly conserved cavity spanned by residues Pro82, Phe83, and Val87 of the ZA loop and Ile146 of helix αC. The slope of the peptide backbone varied for different ligands, as indicated by arrows. The superimposition was performed on the apo form of the bromodomain structures.

butyryl-CoA, so that the abundance of the donor substrate may determine the type of modification.[10–13] Similarly, depropionylation and debutyrylation is mediated by histone deacetylases (HDACs), as shown for sirtuins from bacteria and higher organisms.[11,23] The short-chain acyl modifications of histones may therefore rather be tolerated as metabolic side effects.

Propionyl-CoA is derived from the catabolism of odd-chain fatty acids and amino acids, whereas butyryl-CoA is a metabolic intermediate formed during the β oxidation of fatty acids as well as a substrate for fatty-acid elongation.[10] The concentration of short-chain CoAs thus fluctuates depending on the diet and cellular physiological conditions.[24,25] For example, under conditions of starving, more propionyl-CoA could be produced from the resorption of lipid bodies and subsequently transferred to histone sites. It might be interesting to analyze how a specific diet of fatty acids could influence gene activation, or how defects in the metabolic degradation of lipid bodies could affect histone modification. An equal or decreased binding affinity to bromodomains, as observed in this study, would ensure that the gene-expression machinery is at least partially maintained.

On the basis of our findings, we suggest that BET-family bromodomains recognize histone propionylation, and to a lesser extent butyrylation, similarly to acetylation. Whereas the water shell in the bromodomain recognition site remains untouched, one might envision that the hydrophobic moiety could have a significant contribution at the surrounding surface of the binding site, as observed for K8 in the twin-acetylation motif H4K5acK8ac.[18] A combination of acetylated and propionylated or butyrylated lysine modifications might be well-suited to form such an arrangement with appropriate interactions. However, it remains open whether a different domain may interact with these modifications with much higher affinity and specificity, so that these histone marks could be assigned a different functionality and readout scheme.

Received: May 5, 2010
Published online: August 16, 2010

Keywords: bromodomains · epigenetics · structural biology · transcription · X-ray diffraction

[1] T. Jenuwein, C. D. Allis, *Science* **2001**, *293*, 1074 – 1080.
[2] S. L. Berger, *Nature* **2007**, *447*, 407 – 412.
[3] T. Kouzarides, *Cell* **2007**, *128*, 693 – 705.
[4] C. E. Berndsen, J. M. Denu, *Curr. Opin. Struct. Biol.* **2008**, *18*, 682 – 689.
[5] S. D. Taverna, H. Li, A. J. Ruthenburg, C. D. Allis, D. J. Patel, *Nat. Struct. Mol. Biol.* **2007**, *14*, 1025 – 1040.
[6] A. Dey, J. Ellenberg, A. Farina, A. E. Coleman, T. Maruyama, S. Sciortino, J. Lippincott-Schwartz, K. Ozato, *Mol. Cell. Biol.* **2000**, *20*, 6537 – 6549.
[7] B. Liu, Y. Lin, A. Darwanto, X. Song, G. Xu, K. Zhang, *J. Biol. Chem.* **2009**, *284*, 32288 – 32295.
[8] K. Zhang, Y. Chen, Z. Zhang, Y. Zhao, *J. Proteome Res.* **2009**, *8*, 900 – 906.
[9] Z. Cheng, Y. Tang, Y. Chen, S. Kim, H. Liu, S. S. Li, W. Gu, Y. Zhao, *Mol. Cell. Proteomics* **2009**, *8*, 45 – 52.
[10] Y. Chen, R. Sprung, Y. Tang, H. Ball, B. Sangras, S. C. Kim, J. R. Falck, J. Peng, W. Gu, Y. Zhao, *Mol. Cell. Proteomics* **2007**, *6*, 812 – 819.
[11] J. Garrity, J. G. Gardner, W. Hawse, C. Wolberger, J. C. Escalante-Semerena, *J. Biol. Chem.* **2007**, *282*, 30239 – 30245.
[12] C. E. Berendsen, B. N. Albaugh, S. Tan, J. M. Denu, *Biochemistry* **2007**, *46*, 623 – 629.
[13] H. Leemhuis, L. C. Packman, K. P. Nightingale, F. Hollfelder, *ChemBioChem* **2008**, *9*, 499 – 503.
[14] M. D. Resh, *Nat. Chem. Biol.* **2006**, *2*, 584 – 590.
[15] H. Gerlach, V. Laumann, S. Martens, C. F. Becker, R. S. Goody, M. Geyer, *Nat. Chem. Biol.* **2010**, *6*, 46 – 53.
[16] C. T. Walsh in *Posttranslational Modification of Proteins*, Roberts, Colorado, **2006**.
[17] F. Vollmuth, W. Blankenfeldt, M. Geyer, *J. Biol. Chem.* **2009**, *284*, 36547 – 36556.
[18] J. Morinière, S. Rousseaux, U. Steuerwald, M. Soler-López, S. Curtet, A.-L. Vitte, J. Govin, J. Gaucher, K. Sadoul, D. J. Hart, J. Krijgsveld, S. Khochbin, C. W. Müller, C. Petosa, *Nature* **2009**, *461*, 664 – 668.
[19] D. C. Hargreaves, T. Horng, R. Medzhitov, *Cell* **2009**, *138*, 129 – 145.
[20] S. Mujtaba, L. Zeng, M. M. Zhou, *Oncogene* **2007**, *26*, 5521 – 5527.
[21] C. M. Stegmann, D. Seeliger, G. M. Sheldrick, B. L. de Groot, M. C. Wahl, *Angew. Chem.* **2009**, *121*, 5309 – 5312; *Angew. Chem. Int. Ed.* **2009**, *48*, 5207 – 5210.
[22] A. J. Ruthenburg, H. Li, D. J. Patel, C. D. Allis, *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 983 – 994.
[23] B. C. Smith, J. M. Denu, *J. Biol. Chem.* **2007**, *282*, 37256 – 37265.
[24] M. T. King, P. D. Reiss, *Anal. Biochem.* **1985**, *146*, 173 – 179.
[25] P. P. Halarnkar, G. J. Blomquist, *Comp. Biochem. Physiol. Part B* **1989**, *92*, 227 – 231.

