# Do protein motifs read the histone code?

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**Summary**

**The existence of different patterns of chemical modifications (acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation) of the histone tails led, some years ago, to the histone code hypothesis. According to this hypothesis, these modifications would provide binding sites for proteins that can change the chromatin state to either active or repressed. Interestingly, some protein domains present in histone-modifying enzymes are known to interact with these covalent marks in the histone tails. This was first shown for the bromodomain, which was found to interact selectively with acetylated lysines at the histone tails. More recently, it has been described that the chromodomain can be targeted to methylation marks in histone N-terminal domains. Finally, the interaction between the SANT domain and histones is also well documented. Overall, experimental evidence suggests that these domains could be involved in the recruitment of histone-modifying enzymes to discrete chromosomal locations, and/or in the regulation their enzymatic activity. Within this context, we review the distribution of bromodomains, chromodomains and SANT domains among chromatin-modifying enzymes and discuss how they can contribute to the translation of the histone code. *BioEssays* 27:164–175, 2005.**



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Abbreviations: HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; Chromodomain, chromatin organization modifier; SANT, "SWI3, ADA2, N-CoR and TFIIB B"; NURD, nucleosome-remodelling and deacetylase complex; SAGA, Spt-Ada_Gcn5 acetyltransferase; SUV39H1, suppressor of variegation 3–9 homologue 1; SWI/SNF, switching-defective/sucrose nonfermenting; PCAF, p300/CBP associated factor; NURF, nucleosome remodelling factor, CBP, CREB-binding protein; RSC, remodel the structure of chromatin,; NuA4, nucleosome acetyltransferase of histone H4; SMRT/N-CoR, silencing mediator of retinoid and thyroid receptor/nuclear receptor co-repressor. MLL, mixed lineage leukaemia; E(Z),enhancer of Zeste.

## The histone code hypothesis

The packing of the eukaryotic genome into chromatin provides the means for compaction of the entire genome inside the nucleus. However, this packing restricts the access to DNA of the many regulatory proteins essential for biological processes like replication, transcription, DNA repair and recombination.[1] There are two mechanisms that can counterbalance the repressive nature of chromatin, allowing access to nucleosomal DNA: (i) covalent modification of histone tails like acetylation, methylation, phosphorylation and ubiquitination;[2–5] and (ii) altering of the nucleosomal structure by enzymes utilising energy from ATP hydrolysis.[6]

In the early nineties, it was proposed that histone covalent modifications can work as recognition signals, directing the binding to chromatin of non-histone proteins that determine chromatin function.[7,8] More recently, it has been hypothesized that specific tail modifications and/or their combinations constitute a code, the histone code, that determines the transcriptional state of the genes.[9–11] According to this hypothesis, "multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple tails, specify unique downstream functions".[9]

In the last years, an increasing amount of experimental data has provided clear support for the different aspects of the histone code hypothesis, contributing to refine and improve it.[For review 12,13] One important point that has been addressed by different authors is the idea that the histone code must use combinations of modifications.[9] For example, H3 methylated at K9 could initiate chromatin condensation and silencing[14,15] but, in the context of methylated H3K4 and H4K20, methyl-K9 H3 helps to maintain active marks by allowing the binding of BRAHMA, the enzyme of the remodelling dSWI/SNF complex.[16]

Another aspect of the histone code hypothesis that has received much attention, is the idea that modifications on the same or different histone tails may be interdependent. That is, the fact that modification in one residue can determine that of another either in *cis* or, more surprisingly, in *trans*. In the first case, it has been shown that methylation of H3K4 has two important effects: it blocks both the binding of the remodelling deacetylation complex NURD and the methylation of H3K9, thereby preventing the placement of silencing marks.[17] As an example of trans effect, we can mention that ubiquitination of H2B K123 is required for an efficient methylation of H3K4.[18]

Recently, two new concepts have been introduced to understand the basis of the signalling by combinations of histone modifications, the concepts of ''binary switches'' and ''modification cassettes''.[19] In the former, neighbouring modifications act together, while for the latter residues in linear strings of densely modifiable sites can have different biological readouts, depending on their modification state.

Combinations of modifications appear to be important for both short- and long-term transcriptional regulation, and have been described in different systems. In the first case, there are clear experimental results showing that regulation of rapid transcriptional processes usually requires a cascade of modification events. For example, activation of the IFN-β gene requires acetylation of several lysines in histones H3 and H4 that mediate the recruitment of the SWI/SNF complex and TFIID, respectively.[20] In the case of long-term regulation, there is less experimental evidence, although it is now clear that some specific histone modifications have the potential to exert long-term effects. For example, H3 methylated at K9 has the potential to initiate chromatin condensation and silencing.[14,15] Also, Czermin, Müller and colleagues have shown that H3 methylated by the E(Z) complex, binds specifically to polycomb protein, suggesting a direct relationship between H3 methylation and silencing by PcG complex.[21,22]

Finally, it has to be mentioned that recently the histone code hypothesis has been extended to the nucleosome code hypothesis, by proposing that high-order chromatin is largely dependent on the local concentration and combination of differentially modified nucleosomes.[10]

## Chromatin-binding domains and the translation of the histone code

An important issue when considering the histone code is how it is translated. More precisely, how the combinations of modifications that constitute the code are recognised and then translated into a given functional effect. According to the histone code hypothesis, the histone modification marks would provide the binding sites for a series of effector proteins that would affect chromatin function.[9] Interestingly, histone-modifying enzymes are unable to access their substrates unless targeted there. In other words, the same enzyme will not modify all histones in all genes, at the same time; only that subset of genes that have recruited the modifying enzyme to the promoter will be regulated by it. This highlights the relevance of the targeting process, at the origin of the selectivity of the enzyme action, as an important feature of the regulation by histone modification. Within this context, it is clear that protein domains able to interact with chromatin and/or its modified components—like bromodomains, chromodomains or the SANT domains—can play a crucial role in the targeting process. These domains (or protein modules, as they have been named[19]) could contribute to both the recognition of specific patterns of modifications, as well as to their setting at given locations. Here, we explore this idea by analysing the distribution of chromatin-binding domains (bromodomain, chromodomain and SANT domain) among chromatin-modifying enzymes (HAT, HDAC, HMT and ATP-dependent remodelling enzymes). To this end, we first review the main structural and functional characteristics of the chromatin-binding domains, together with their distribution among chromatin-modifying enzymes. Then, on that basis we consider their possible role in the translation of the histone code, as targeting elements of the histone-modifying enzymes in the context of short-term regulation. Finally, we discuss their role in the long-term regulation processes.

## The bromodomain

Bromodomains are small protein domains that form an extensive family.[23] The first reported bromodomain was found in the *Drosophila* Brahma protein.[24] Bromodomains were later found in many chromatin-associated proteins and most histone acetyltransferases.[25,26]

### *Structure and function*

The three-dimensional structure of a prototypical bromodomain from the histone acetyltransferase PCAF shows an unusual left-handed four-helix bundle (Fig. 1A).[27,28] A long loop between helices α Z and α A is packed against the loop connecting helices B and C to form a surface-accessible hydrophobic pocket, located at one end of the four-helix bundle. This unique feature is conserved in the bromodomain family and can be seen in the bromodomain structure of human GCN5, *S. cerevisiae* GCN5p and human $TAF_{II}250$.[29–31]

The bromodomain role in chromatin remodelling was suggested some time ago, on the basis of yeast genetic studies.[24] However, its biological function was confirmed after the more recent discovery that bromodomains function as acetyl-lysine binding domains.[28–31] Initially, in vitro studies showed that bromodomains preferentially bind acetylated peptides, leading to speculation that acetylated histone tails could become targets for the binding of bromodomain-containing proteins.[28,30–32] This has been recently confirmed by Hassan and colleagues, who have shown that the SWI/SNF complex is retained to the chromatin by previous histone acetylation by SAGA or NuA4, and after removal of the

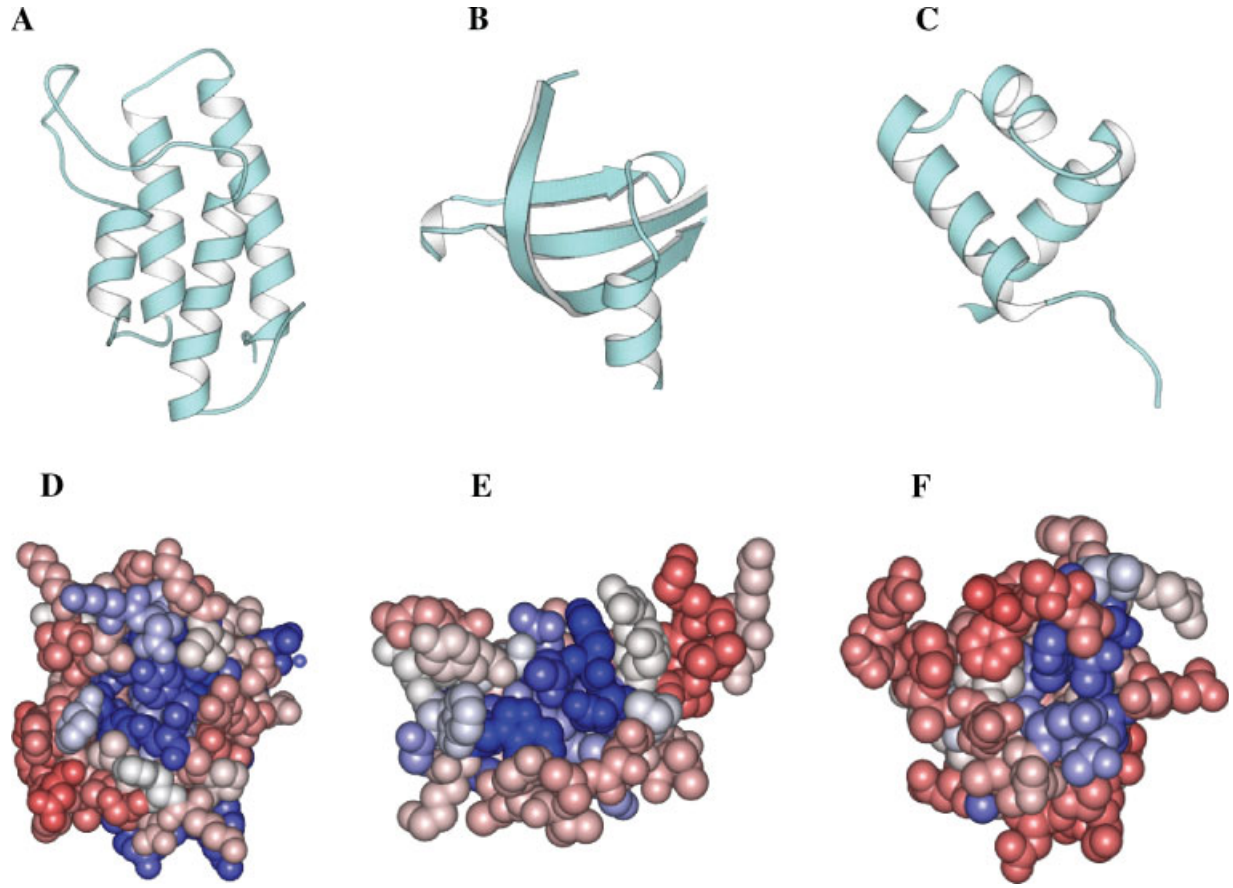


**Figure 1. A–C:** Domain structures. MOLSCRIPT[87] figures of the structures of the bromodomain (A), chromodomain (B) and SANT domain (C), PDB codes: 1N72, 1PFB and 1OFC, respectively. **D–F:** Conservation at the domain binding sites. The respective binding sites of each domain, coloured according to the residue conservation degree, derived from the multiple sequence alignment for each domain family. We measured the conservation degree utilising the Shannon entropy at each column position of the multiple sequence alignment.[88,89] The resulting values were mapped to the residues in the structure of the corresponding domain, utilising a colour code that goes from red (highly variable residue) to blue (highly conserved residue). The figures were obtained with the program Insight II, from Accelrys. Pfam[90] multiple sequence alignments were utilised for the bromodomain (D)and chromodomains (E) and SMART[91] multiple sequence alignment for the SANT domain (F) (not available in Pfam). The latter was edited to eliminate obvious members of the MYB-family transcription factor, more likely to belong to a different functional family.[72]

transcription factor (Gal4-VP16).[33] The retention requires the bromodomain of Swi2/Snf2 subunit of the SWI/SNF complex.[33] Further, the SAGA complex itself is anchored to acetylated arrays, following removal of the activator, and can coordinate nucleosomal remodelling; however, this will only happen if the bromodomain of the Gcn5 subunit is intact, providing a self-perpetuating mark tethered to a small chromatin domain.[34]

If bromodomains play a role in enzyme targeting to the chromatin, then one would expect a high conservation degree at their binding sites independently of the chromatin-modifying enzyme carrying them. In Fig. 1D, we plot a view of the binding site, with all residues coloured according to their conservation degree, as derived from the multiple sequence alignment for the domain family. In accordance with the proposed role, we observe that highly conserved residues tend to define, or cluster around, the domain binding site.

Overall, these data confirm that the bromodomain has the ability to bind acetylated histone tails in vivo, with an apparent independence of the protein to which it belongs, and this ability can be utilised by different chromatin-remodelling enzymes to find and/or act on their targets.

### *Distribution among chromatin-modifying enzymes*

In accordance with the above mentioned functional data, we find that the bromodomain is widely distributed among the different enzymes that acetylate, methylate or remodel chromatin (Table 1).

**Table 1.** Summary table of the HAT-, HMT-, and ATP-dependent chromatin-remodelling enzymes carrying bromodomains, chromodomains or SANT domains as part of their sequence, in different organisms

| Protein activity | Domain | Organism | Gene | # Domains | Residue specificity | | | Molecular function |
|---|---|---|---|---|---|---|---|---|
| | | *D. melanogaster* | BRM | 1 | | | | *Transc. coactiv./corepres.* |
| *Chromatin Remodeling* | BROMO | *H. sapiens* | BRM | 1 | | | | *Transc. coactiv./corepres.* |
| | | | BRG1 | 1 | | | | *Transc. coactiv./corepres.* |
| | | *S. cerevisiae* | SWI2/SNF2 | 1 | | | | *Transc. coactiv./corepres.* |
| | | | STH1 | 1 | | | | *Transc. coactiv./corepres.* |
| | | *A. thaliana* | GCN5 | 1 | — | | | *Transc. coactiv.* |
| | | *C. elegans* | 1E91 | 1 | — | | | *Transc. coactiv.* |
| | | | CBP-1 | 1 | — | | | *Transc. coactiv.* |
| | | *H. sapiens* | P300 | 1 | H3: K14, K18 | H4: K5, K8 | H2A, H2B | *Transc. coactiv.* |
| | | | PCAF | 1 | H3: K14 | H4: K8 | | *Transc. coactiv.* |
| *Histone Acetyltransferase* | BROMO | | CBP | 1 | H3: K14, K18 | H4: K5, K8 | H2A, H2B | *Transc. coactiv.* |
| | | | TAF250 | 2 | H3: K14 | H4 | H2A | *Transc. coactiv.* |
| | | | GCN5 | 1 | H3: K14 | H4: K8, K16 | | *Transc. coactiv.* |
| | | *M. musculus* | GCN5 | 1 | H3: K14 | H4: K8, K16 | | *Transc. coactiv.* |
| | | | PCAF | 1 | H3: K14 | | | *Transc. coactiv.* |
| | | | CBP | 1 | H3: K14, K18 | H4: K5, K8 | H2A, H2B | *Transc. coactiv.* |
| | | | LOC330129 | 1 | — | | | *Unknown* |
| *Histone Methyltransferase* | BROMO | *H. sapiens* | MLL | 1 | H3: K4 | | | *Transc. coactiv.* |
| | | *A. thaliana* | CHD-3 | 2 | | | | *Transc. corepres.* |
| | | *C. elegans* | LET-418 (MI-2 like) | 2 | | | | *Transc. corepres.* |
| | | | CHD-3 | 2 | | | | *Transc. corepres.* |
| *Chromatin Remodeling* | CHROMO | *D. melanogaster* | CHD-3 | 2 | | | | *Transc. corepres.* |
| | | *H. sapiens* | CHD-3 | 2 | | | | *Transc. corepres.* |
| | | | CHD-4 | 2 | | | | *Transc. corepres.* |
| | | | CHD-5 | 2 | | | | *Unknown* |
| | | *O. sativa* | P0018C10.33 | 2 | | | | *Unknown* |
| | | *H. sapiens* | CDY-1 | 1 | — | | | *Transc. corepres.* |
| | | | CDY-2 | 1 | — | | | *Transc. corepres.* |
| | | | TIP60 | 1 | H2A: K5 | H3: K14 | H4: K5, K8, K12, K16 | *HIV tat interaction* |
| *Histone Acetyltransferase* | CHROMO | | MORF4L1 | 1 | H4: K5, K8, K12, K16 | | H3 | *Transc. coactiv.* |
| | | *D. melanogaster* | CG6121 | 1 | — | | | *Unknown* |
| | | *M. musculus* | MORF4L1 | 1 | — | | | *Unknown* |
| | | *R. norvegicus* | TIP60 | 1 | H2A: K5 | H3: K14 | H4: K5, K8, K12, K16 | *HIV tat interaction* |
| | | *S. pombe* | SPAC637.12C (Myst family) | 1 | — | | | *Unknown* |
| | | *S. cerevisiae* | ESA1 | 1 | H4: K5, K8, K12, K16 | H3 | H2A | *Cell Cycle Regulator* |
| | | *D. melanogaster* | SU(VAR)3-9 | 1 | H3: K9 | | | *Heterochr. Formation/Transc. corepres.* |
| | | *H. sapiens* | SUV39H1 | 1 | H3: K9 | | | *Heterochr. Formation/Transc. corepres.* |
| | | | SUV39H2 | 1 | H3: K9 | | | *Heterochr. Formation/Transc. corepres.* |
| *Histone Methyltransferase* | CHROMO | *M. musculus* | SUV39H1 | 1 | H3: K9 | | | *Heterochr. Formation/Transc. corepres.* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | SUV39H2 | 1 | H3: K9 | | | *Heterochr. Formation/Transc. corepres.* |
| | *R. norvegicus* | SUVAR39-1 | 1 | H3: K9 | | *Heterochr. Formation/Transc. corepres.* |
| | | SUVAR39-2 | 1 | H3: K9 | | *Heterochr. Formation/Transc. corepres.* |
| | *S. pombe* | CLR4 | 1 | H3: K9 | | *Heterochr. Formation/Transc. corepres.* |
| | *S. cerevisiae* | ISW1 | 2 | | | *Transc. coactiv./corepres.* |
| | | ISW2 | 2 | | | *Transc. coactiv./corepres.* |
| *Chromatin Remodeling* | *D. melanogaster* | ISWI | 2 | | | *Transc. coactiv./corepres.* |
| | *H. sapiens* | SNF2L | 2 | | | *Transc. coactiv./corepres.* |
| | | SNF2H | 2 | | | *Transc. coactiv./corepres.* |
| | *A. thaliana* | MEDEA | 1 | — | | *Gene Silencing* |
| | | EZA1 | 1 | — | | *Gene Silencing* |
| | *D. melanogaster* | E(Z) | 2 | H3: K27 | | *Gene Silencing* |
| | *H. sapiens* | EZH1 | 2 | H3: K27 | | *Gene Silencing* |
| *Histone Methyltransferase* | SANT | EZH2 | 2 | — | | *Gene Silencing* |
| | *M. musculus* | EZH1 | 2 | H3: K27 | | *Gene Silencing* |
| | | EZH2 | 2 | — | | *Gene Silencing* |
| | *Z. mays* | EZ1 | 1 | — | | *Gene Silencing* |
| | | EZ2 | 1 | — | | *Gene Silencing* |
| | | EZ3 | 1 | — | | *Gene Silencing* |

### *Bromodomain and HAT enzymes*

The bromodomain is present in the members of *Arabidopsis*, human and mouse GCN5/PCAF family, human and mouse CBP/p300, human $TAF_{II}250$, TAF1L, acetyltransferases and two putative HAT enzymes: Q9N3N7 (involved in membrane transport in *C. elegans*) and LOC330129 in mouse, which encodes for a protein similar to PCAF. The presence of bromodomains in many HAT enzymes suggests that self-perpetuation of the HAT at acetylated locus through interactions between their bromodomains and acetylated histones could be a common feature for these enzymes (Fig. 2B).

### *Bromodomain and HMT enzymes*

Bromodomains are also part of some HMT enzymes: Ash1, RIZ (member of the RIZ family), and MLL (members of the TRX proteins). MLL also has an MBD domain that may recognise methylated DNA.[35]

### *Bromodomain and ATP-dependent remodelling enzymes*

Remodelling enzymes that utilise ATP to alter chromatin structure also have bromodomains: SNF2α and SNF2β from human, as well as their homologous SNF2 in *Saccharomyces cerevisiae* and Brahma in *Drosophila*, all of them members of the SWI/SNF complexes, and STH1, subunit of the yeast RSC complex.

Bromodomains can also be found in subunits, with no catalytic activity, of remodelling complexes, where they could help the latter in the recognition of previously modified chromatin or to stabilize the interaction of the complex. For example, bromodomains can be found in (1) *C. elegans*, human, mouse and rat MTA-1 protein, subunit of the MTA-1 complex, (2) in human and mouse ACF1, subunit of the CHRAC complex, (3) in yeast spt7, component of the SAGA complex, and (4) in yeast RSC1 and 2, components of the RSC complex. In the latter, the bromodomain is essential for the RSC remodelling function, although it is not required for complex assembly.[36]

Taken together, these data suggest that specific interactions between some ATP remodelling enzymes and chromatin could be stabilised by the bromodomain Ac-lys interaction, helping to establish the final remodelled chromatin structure, in accordance with work by Agaliote and colleagues.[20]

## The chromodomain

The chromodomain was first identified as a common domain between two distinct regulators of chromatin structure in *Drosophila*: HP1 and Polycomb.[37] Later, chromodomains have been found in many other chromatin regulators: (i) remodelling factors involved in causing conformational changes by ATP-dependent movement of nucleosomes and (ii) histone acetyltransferases and methyltransferases.[38,39]

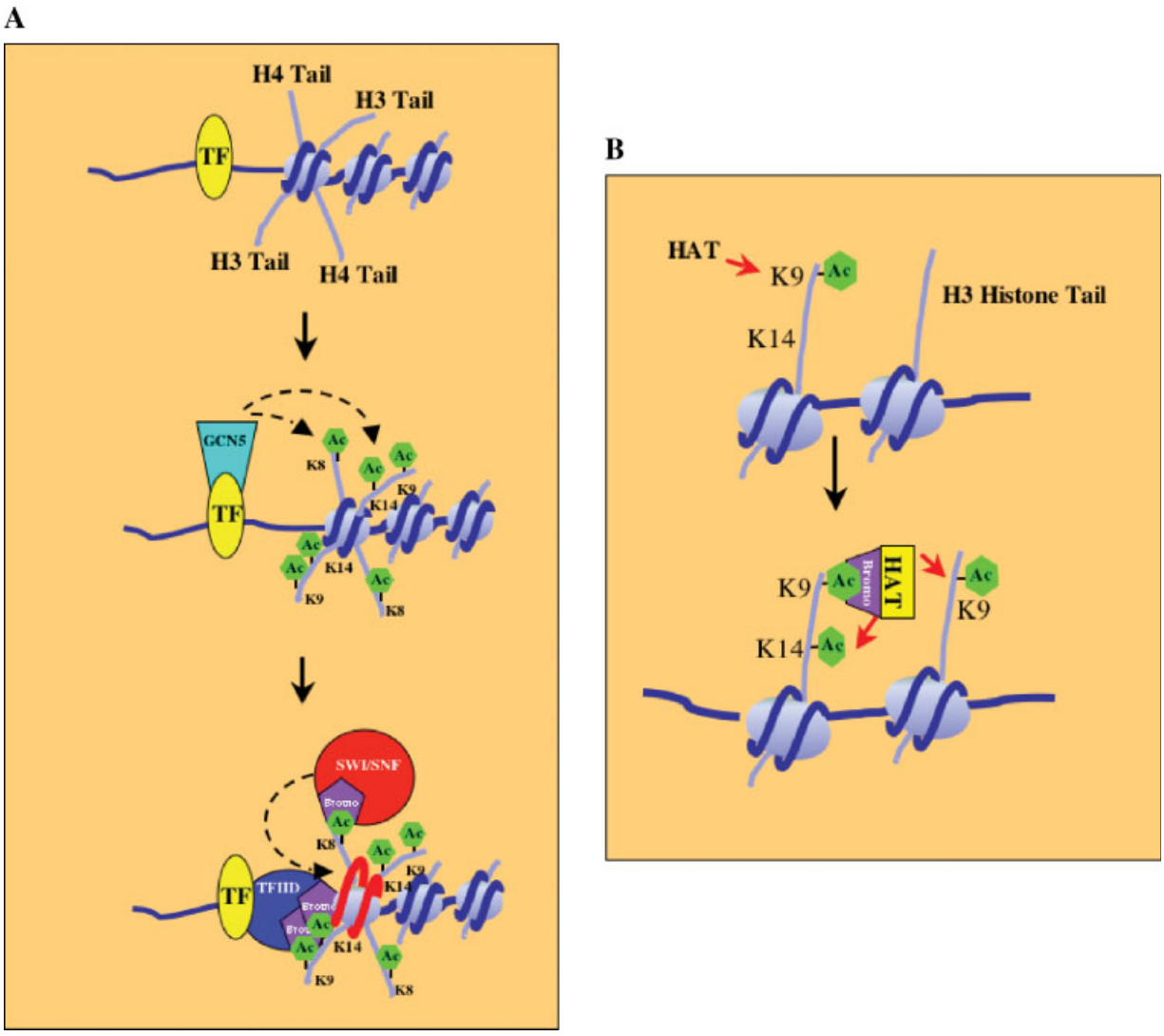


**Figure 2.** Model for cooperation of the chromatin modifying enzymes and the chromatin-binding domains (illustrated for the case of bromodomains). **A:** Possible functional interactions between acetylated histone tails and bromodomains containing enzymes that lead to a cascade of events to activate transcription. GCN5 acetyltransferase is recruited to the gene promoter by interaction with a transcription factor. GCN5 acetylates H3K9, H3K14 and H4K8. Finally, the bromodomain-containing transcription complexes SWI/SNF and TFIID are recruited to the promoter by specific interactions between their bromodomain and specifically acetylated histone tails. **B**: Possible positive feedback in chromatin signalling mediated by specific interactions between acetylated histone tails and HAT enzymes containing bromodomains that leads to self-perpetuation of activating marks on chromatin.

*Structure and function*

The structure of the HP1 chromodomain, in complex with a peptide from histone 3 with Lys 9 methylated, consists of a three-stranded antiparallel β-sheet supported by an α-helix that runs across the sheet (Fig. 1B).[40] The binding pocket for the N-methyl group is provided by three aromatic side chains that become ordered on binding of the peptide.[28,41,42]

The finding of the chromodomain in two completely different epigenetic repressors, like HP1 and Polycomb, immediately suggested that chromodomains can have chromatin-related functions.[37,43] Although the role of the chromodomain within these proteins is not yet fully understood, experimental evidence points to an involvement in protein–protein interactions.[40,44] In particular, recent work from different laboratories has shown that the HP1 chromodomain can recognise methylation of Lys 9 in histone H3, thus directing the binding of other proteins to control chromatin structure and gene expression.[14,15,45] The structure of the chromodomain

complex with a histone H3 peptide that includes methylated Lys 9 explains how the binding can take place, with the lysine side chain almost fully extended and surrounded by residues conserved in many chromodomains.[40] The latter is particularly relevant, as it would support the chromatin-binding ability of chromodomains independently of the protein to which they belong.

It has to be mentioned that, apart from recognising methyl-lysines, chromodomains can also serve to DNA and/or RNA recognition.[41,42,46–48] For example MOF and MSL-3 use their chromodomains to bind the non-coding roX RNA, crucial for the integrity and targeting of the *Drosophila* dosage compensation complex to the X chromosome;[49–51] this situation is similar to the role of the HP1 chromodomain in heterochromatin formation, but recognizing RNA instead methyl-Lys. It is interesting to notice that it has been suggested that non-coding RNAs could be involved in some epigenetic regulations and some enzymes, as the chromodomain-containing enzyme SUV39 that trimethylates H3K9, requires an unidentified RNA.[52]

As mentioned before, the chromodomain is also able to bind DNA. For example, the chromodomain of Mi-2 binds the nucleosome but, surprisingly, deletion of all histone tails does not eliminate such an interaction.[47] The latter is maintained thanks to a sequence unspecific binding to the nucleosomal DNA.[47] In contrast to the methyl-lysine binding (see above), structural determinants indicative of RNA- or DNA-binding chromodomain have not yet been identified, although two point mutations on the MOF chromodomain eliminate binding to RNA in vitro.[48]

As for bromodomains, we have looked at the binding site conservation degree of chromodomains (Fig. 1E). We find a substantial conservation degree, supporting a similar role in enzyme targeting for these domains.

The previous data support the idea that chromodomains, like bromodomains, are able to identify and bind specific tags in histones, in particular methylated lysines, and this binding would be vital to recruit different chromatin-modifying enzymes to their targets.

It has also been reported that the chromodomain of HP1 can interact with histone H3 in the absence of the N-terminal tail; however, while this interaction may contribute to chromatin binding in general, it does not explain the specific targeting of HP1.[53]

### *Distribution among chromatin-modifying enzymes*

The distribution of the chromodomain among these enzymes is more restricted than that of the bromodomain. However, we have found it in HAT, HMT and ATP-dependent chromatin-remodelling enzymes (Table 1).

### *Chromodomain and HAT enzymes*

Chromodomains can be found in human and mouse MORF4L1, identified on the basis of the ability of these proteins to induce replicative senescence in immortal human cell lines.[54] The members of this family have a clear similarity to Msl-3 and Eaf3p, both known components of multisubunit histone acetyltransferase complexes.[55] Msl-3 is a component of the dosage compensation complex that acetylates histone H4 on the male X chromosome at multiple sites.[56] Eaf3p is a component of the yeast NuA4 HAT complex that carries the Esa1p HAT protein.[57] This complex also functions by specifically acetylating histone H4 in vivo and has been linked to transcriptional activation and nucleosome remodelling in yeast and flies.[58,59]

The chromodomain is also present (1) in rat and human Tip60, and *Saccharomyces pombe* SPAC637.12C proteins, members of the Myst family of HAT, and (2) in human and mouse CDY1 and 2 proteins, which contain a putative HAT domain and are components of the heterochromatin-like complexes that act as gene repressors during spermatogenesis.[60,61]

### *Chromodomain and HMT enzymes*

The chromodomain can be found in different HMT enzymes, in the members of the suvar-3-9 family in *Drosophila* [su(var)3-9], yeast (clr4), insect [su(var)3-9] and mammalian [su(var)3-9H1 and H2].[62]

### *Chromodomain and ATP-dependent remodelling enzymes*

Chromodomains are found in (1) members of the SNF2/RAD54 helicase family (CHD3 in *Arabidopsis thaliana, Drosophila* and *C. elegans*),[63–65] (2) Mi-2 and human Mi-2$\beta$ or CHD4,[66–68] (3) Mi-2$\alpha$ or CHD3,[68] and (4) CHD5.[69] Mi-2 enzyme remodels chromatin thanks to its ATP hydrolysis ability, and is part of a complex called NURD[55] that can also deacetylate histones. This suggests that NURD could be specifically targeted to previously methylated chromatin through the chromodomain.

Finally, a chromodomain can also be found in a putative remodelling enzyme, Q8LJJ7, from *Oryza sativa*.

## The SANT domain

The SANT domain was identified as a small motif, approximately 50 amino acids, present in nuclear receptor co-repressors. Sequence and structure analysis show a clear similarity between the SANT domain and the DNA-binding domain (DBD) of c-Myb related proteins.[70]

### *Structure and function*

The SANT domain consists of three $\alpha$-helices, each with a bulky aromatic residue, arranged in a helix-turn-helix motif (Fig. 1C).[71] The overall structure is similar to that of Myb DBD, although the SANT domain is functionally divergent from the canonical Myb DBD.[72] The SANT domain is present in some ATP-dependent remodelling enzymes complexes: yeast Swi3p, Rsc8p, BAF155/170 and *Drosophila* ISWI. It has been

shown that the SANT domain is essential for the in vivo functions of yeast Swi3p, Ada2p and Rsc8p, subunits of three chromatin-remodelling complexes. The general role of the SANT domain is to stabilise, through direct binding, histone N-terminal tails in a conformation favouring their binding to the modifying enzymes, and the subsequent catalytic process.[73,74] Although the SANT domain interacts primarily with unmodified histone tails, we decided to include it here because (1) it has a central role in chromatin remodelling, being the unique histone-interaction module that couples histone binding to enzyme catalysis, and (2) it is present, like bromodomains and chromodomains, in the two enzyme classes responsible of chromatin modifications (enzymes that catalyse the histones covalent modifications and complexes using ATP hydrolysis). The preference of the SANT domain for unmodified histone tails suggests that histone deacetylation could increase its affinity for histone tails. Interaction with unacetylated histone tails could block the binding of HATs, thus maintaining the deacetylated state, as proposed by Yu and colleagues.[75]

When looking at the conservation degree of the SANT domain residues (Fig. 1F) we find a less-clear trend than for bromo or chromodomains. While significant, the conservation degree of binding site residues is smaller in this case; this can be attributed to the high functional degeneracy of the underlying family (see above), and to the less specific nature of its binding.

### *Distribution among chromatin-modifying enzymes*

The SANT domain is broadly present among ATP-dependent remodelling enzymes and their complexes, but it can also be found in HMTs (Table 1), and in proteins forming part of complexes with HAT and HDAC activities, thus suggesting an important role in regulating chromatin accessibility.

### *SANT domain and HAT enzymes*

We have not found any HAT or HDAC enzyme with a SANT domain as part of its sequence, although some components of HAT and HDAC complexes can have it: (1) SPR1 from *C. elegans*, part of the Co-REST corepressor complex, essential for HDAC1 activation,[76] (2) human and mouse N-CoR, that interact with HDAC7 and together with Sin3 and HDAC,[77] and (3) ADA2 proteins, ADA2$\alpha$ and ADA2$\beta$ in yeast, mouse, rat and human. The latter deserve further mention, as ADA2 proteins form part of the SAGA, ADA/GCN5 and PCAF histone acetyl-transferase complexes. Interestingly, it has been observed that ADA and SAGA complexes containing a deletion of the ADA2 SANT domain show a reduced ability to bind non-acetylated histone tails, being inactive in nucleosomal HAT assays.[73,78]

SANT domains are also present in several subunits of other co-repressor complexes that possess HDAC activity, such as MLL and SMRT. In the latter, the SANT domain functions as a histone–tail interaction domain that binds to non-acetylated histone H4 peptides.[75] In addition, the presence of the SANT domain enhances the HDAC activity of the SMRT–HDAC3 complex, by increasing the affinity of the latter for histone tails.[75]

### *SANT domain and HMT enzymes*

SANT domains can be found in several members of the polycomb group of proteins, involved in the repression of homeotic genes and with HMT activity: MEDEA and EZA1 in *Arabidopsis*, EZ1-3 in maize, EZ in *Drosophila*, and EZH1-2 in both mouse and human.

### *SANT domain and ATP-dependent remodelling enzymes*

Some ATP-remodelling enzymes have a SANT domain: *Drosophila* ISWI, (the catalytic subunit of the remodelling complex NURF, CHRAC and ACF), yeast ISWI1 and ISWI2 (catalytic subunits of ISWI1 and ISWI2 complexes, respectively) and human SNF2L and SNF2H (member of the RSF complex). The SANT domain is also present in: yeast Swi3, a component of the SWI/SNF complex; human and mouse MTA1 and MTA2, which are part of the remodelling, deacetylating, complexes NURD, RSC8p and BAF155/170. The presence of the SANT domain in these enzymes suggests that targeting, or stabilization, of the chromatin–enzyme interaction could happen frequently by direct interaction between the SANT domain and the chromatin component. This would help coupling the histone-tail binding and enzymatic activity, as has been previously suggested by Boyer and colleagues.[73,74]

All of these data suggest that the SANT domain can mediate interactions between remodelling enzymes and their chromatin substrates. More precisely, the SANT domain could contribute: (1) to the recruitment of chromatin modifying enzymes, or (2) to help the interaction between histones and the enzymes. As mentioned before, the latter would follow from the SANT–histone interaction that would improve the histone binding and subsequent catalysis by the modifying enzymes.[73]

## What is the role of chromatin-binding domains?

The data previously discussed (Table 1) show that bromodomains, chromodomains, and SANT domains can be found in the three chromatin-modifying enzymes, or their complexes, although with an unequal distribution among them. While in some cases the enzyme may carry more than one copy of the same domain (Table 1), no combination of different domains has been found (Table 1). Overall, the differential distribution of the chromatin-binding domains among the chromatin-modifying enzymes (Table 1) is in accordance with the idea that these domains can confer specific chromatin-binding properties to the different enzyme families. For example, acetylation at H4K8 could help the recruitment of the

remodelling complex, through interaction with the BRG1 bromodomain, thus contributing to prepare chromatin to be transcribed (Fig. 2A);[20] however, this would not be the case for chromatin-remodelling enzymes lacking bromodomains. Or, in the same way, acetylation at H3K14 could help recruitment of some bromodomain-carrying HMTs, that could set a specific combination of activation marks at a given locus, correlated with transcriptional activation, as would be the case for MLL that methylates at H3K4.[79] However, bromodomain-lacking HMTs, which methylate other positions (as Suvar39 at H3K9) and are involved in silencing, will not be targeted to that specific locus.

Within this context, the histone-binding specificity of domains becomes an important issue that deserves further comment. In particular, a critical question is why domains recognize specifically some modified lysines and not others. We discuss below three main contributions to domain binding specificity: (1) sequence variability at the domain binding site, and neighbouring residues, (2) domain copy number, and (3) allosteric changes induced by protein–protein interactions after chromatin binding.

As we have seen before, there is a substantial degree of sequence conservation at the domains binding site (Fig. 1D–F), supporting the overall conservation of function. However, sequence conservation is not complete and some variability is observed for the different domains that could modulate the domain-binding specificity. This can be illustrated by considering the case of bromodomains and chromodomains. For these two domain types, it seems that not all of them, or their acetylated or methylated targets behave similarly. In the case of chromodomains, swapping experiments have shown a non-uniform functional conservation of this domain in silencing assays.[80,81] For example, chromodomain of HP1 recognizes methylated H3K9, while the chromodomains from polycomb (M33) and Mi2 do not bind tightly to methylated lysine residues,[14] probably they are able to recognize other chromatin targets, as DNA or RNA. A similar situation is found for bromodomains, for example, the bromodomain in BRG1 binds the H4 tail acetylated at K8 and bromodomain of Brd2 interacts with acetylated H4K12,[20,82] whereas the double bromodomain in $TAF_{II}250$ binds the H3 tail acetylated at K9 and K14 (Fig. 2A).[20,31] In the case of the budding yeast SAGA HAT complex, Gcn5 and Spt7 subunits contain bromodomains capable of binding acetyl-lysines. However, while the Gcn5 bromodomain is essential for tethering SAGA to acetylated nucleosomes arrays in vitro, the bromodomain of Spt7 is dispensable. However, if swapped into Gcn5 subunit, the Spt7 bromodomain is capable of anchoring SAGA.[33] The latter suggests that specificity of chromatin-binding domains could depend, at least in part, on the protein context. In this particular case, it seems likely that amino acids flanking acetyl-lysines, as well as non-conserved amino acids in and around the bromodomain, could modulate the binding specificity of the latter.

The presence of more than one chromatin-binding domain can also be critical to determine domain-binding specificity. The data in Table 1 show that chromatin-modifying enzymes contain only one type of chromatin recognition motifs (Table 1). However, the number of domain copies may change, and some histone-modifying enzymes contain two tandem copies of the bromodomain, chromodomain or SANT domain. This domain duplication could contribute to the binding specificity, by increasing the stability of the enzyme-dimodified histone, when the modifications are appropriately spaced in the histone tail. Actually, this is the case for the $TAF_{II}250$ double bromodomain that binds to diacetylated H3 at K9 and K14.[31]

Finally, allosteric changes induced upon association with transcription factor complexes, and after interaction with the modified chromatin, can also determine domain binding specificity. For example SWI/SNF is recruited to the promoter through the association of the BRG1 bromodomain with the CBP-acetylated H4K8 tail.[20] The interaction with other acetylated residues in H3 or H4 may be possible in vitro; however, these interactions will not have sufficient strength to ensure stable binding of SWI/SNF to CBP and the promoter.[20]

## Histone-binding domains and long-term regulation

The ideas discussed above can explain how combinations of histone modifications and the chromatin-binding domains that recognize them could regulate short-term transcription. However, they do not completely address the critical issue of what is their contribution to the establishment and maintenance, and even the heritability, of long-term transcriptional states. At present, it is well established that histone modifications have the potential to exert long-term effects, for example H3 methylated at K9 could initiate chromatin condensation and silencing, in part due to its ability to bind proteins such as HP1 through their chromodomains.[14,15] However, the potential of a single modification to have such long-term effects depends on the chromatin context in which the modification is present. For example, in the context of H3K4 and H4K20 methylated nucleosome, methyl-K9 H3 allows the binding of BRAHMA—the enzyme of the remodelling dSWI/SNF complex, a mark to maintain a long-term transcriptionally active locus.[16]

It has been proposed that chromatin-binding domains could play a central role in helping to establish and maintain long-term transcriptional states.[83] This would be due to the ability of some enzymes to form self-sustaining marks in chromatin, by stably binding their enzymatic products through the chromatin-binding domains (Fig. 2B).[83] This stable binding would in turn allow silencing and/or activating complexes to self-perpetuate their potential. For example, many HAT enzymes bind preferentially to acetylated peptides, in vitro binding assays, using their bromodomains.[84] More

particularly, it has been found that the SAGA complex remains anchored to acetylated arrays of nucleosomes through a GCN5 bromodomain, even after removal of the transcription factor VP16, thus providing a self-perpetuating activating mark on chromatin domain.[33] In contrast the NuA4 complex, which lacks a bromodomain, is not retained following removal of the activators.[33]

In the same way, Czermin, Müller and colleagues have shown that Enhancer Zeste, (E(Z)), has methyltransferase activity[21,22] and H3 methylated by the E(Z) complex binds specifically to polycomb protein, suggesting a direct relationship between H3 methylation by E(Z) and assembly of the PcG silencing complex.[21]

While these data suggest a role for chromatin-binding domains in the long-term transcriptional regulation, it is also quite clear that long-term transcriptional regulation is a complex process, requiring a subtle coordination between histone modifications, DNA methylation and binding of silencing RNA.[85,86]

## Conclusions

The histone code hypothesis provides a useful conceptual framework for understanding how gene expression is modulated through covalent marks in chromatin. Particularly, experimental data published during these last years reinforce the idea that the functional effect (activation or repression) of the translation of the histone code will depend (1) on the combination of histone marks laid down by the enzymes recruited to the gene (by a transcription factor or bromo/chromo/SANT interactions), and (2) also on the chromatin architecture of each gene.

Within this context, an issue that remains open is how chromatin-modifying enzymes are targeted to their histone templates. Recent studies show that some domains, able to specifically recognise histones—chromo, bromo and SANT domains—are also present in different chromatin-modifying enzymes—HAT, HMT and ATP-dependent remodelling enzymes—leading to the proposal that they could contribute to the targeting of histone-modifying enzymes to chromatin targets. Here we have reviewed the distribution of chromatin-binding domains among chromatin-modifying enzymes, finding that it is unequal and supporting the idea that these domains can confer specific chromatin-binding properties to the different enzyme families. In addition, we discuss how factors such as sequence variability, domain copy number and allosteric changes can contribute to modulate the domain-targeting properties.

## References


1. Konberg RD, Lorch Y. 1999. Chromatin-modifying and remodelling complexes. Curr Opin Genet Dev 9:148–151.
2. Hebbes TR, Thorne AW, Crane-Robinson C. 1988. A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 7:1395–1402.
3. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, et al. 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406:593–599.
4. Wu J, Grunstein M. 2000. 25 years after the nucleosome model: Chromatin modifications. Trends Biochem Sci 25:619–623.
5. Berger S. 2002. Histone modifications in transcriptional regulation. Curr Opin Genet Dev 12:142–148.
6. Kingston RE, Narlikar GJ. 1999. ATP-dependent remodelling and acetylation as regulators of chromatin fluidity. Genes Dev 13:2339–2352.
7. Turner BM, Birley AJ, Lavender J. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. Cell 69:375–384.
8. Turner BM. 1993. Decoding the nucleosome. Cell 75:5–8.
9. Strahl BD, Allis CD. 2000. The language of covalent histone modifications. Nature 403:41–45.
10. Jenuwein T, Allis CD. 2001. Translating the histone code. Science 293: 1074–1080.
11. Spotswood HT, Turner BM. 2002. An increasingly complex code. J Clin Invest 110:577–582.
12. Turner BM. 2002. Cellular memory and the histone code. Cell 111:285–291.
13. Marmorstein R. 2001. Protein modules that manipulate histone tails for chromatin regulation. Nature Rev 2:422–432.
14. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, et al. 2001. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410:120–124.
15. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410:116–120.
16. Beisel C, Imhof A, Greene J, Kremmer E, Sauer F. 2002. Histone methylation by Drosophila epigenetic regulator Ash1. Nature 419:857–862.
17. Zegerman P, Canas B, Pappin D, Kouzarides T. 2002. Histone H3 lysine 4 methylation disrupts binding of nucleosome remodelling and deacetylase (NuRD) repressor complex. J Biol Chem 227:11621–11624.
18. Sun Z-W, Allis CD. 2002. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature 418:104–108.
19. Fischle W, Wang Y, Alis CD. 2003. Binary switches and modification cassettes in histone biology and beyond. Nature 425:475–479.
20. Agalioti T, Chen G, Thanos D. 2002. Deciphering the transcriptional histone acetylation code for a human gene. Cell 111:381–392.
21. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, et al. 2002. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 111:185–196.
22. Müller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, et al. 2002. Histone methyltransferase activity of a Drosophila polycomb group represor complex. Cell 111:197–208.
23. Jeanmougin F, Wurtz J-M, Douarin B, Chambon P, Losson R. 1997. The bromodomain revisited. TIBS 22:151–153.
24. Tankum JW, Deuring R, Scott MP, Kissinger M, Pattatucci AM, et al. 1992. brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell 68:561–572.
25. Horn PJ, Peterson CL. 2001. The bromodomain: a regulator of ATP-dependent chromatin remodelling? Front Biosci 6:D1019–D1023.
26. Zheng L, Zhou M. 2002. Bromodomain: an acetyl-lysine binding domain. FEBS Lett 513:124–128.
27. Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, et al. 1999. Structure and ligand of a histone acetyltransferase bromodomain. Nature 399: 491–496.
28. Bottomley M. 2004. Structures of proteins that create or recognize histone modifications. EMBO Rep 5:464–469.
29. Hudson BP, Martinez-Yamout MA, Dyson HJ, Wright PE. 2000. Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. J Mol Biol 304:355–370.
30. Owen DJ, Ornaghi P, Yang J-C, Lowe N, Evans PR, et al. 2000. The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase Gcn5p. EMBO J 19:6141–6149.
31. Jacobson RH, Landurner AG, King DS, Tjian R. 2000. Structure and function of a human $TAF_{II}250$ double bromodomain module. Science 288:1422–1425.

32. Winston F, Allis CD. 1999. The bromodomain: a chromatin-targeting module? Nat Struct Biol 6:601–604.
33. Hassan AH, Prochasson P, Neely KE, Galasinski SC, Chandy M, et al. 2002. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell 111:369–379.
34. Syntichaki P, Topalidou I, Thireos G. 2000. The Gcn5 bromodomain co-ordinates nucleosome remodelling. Nature 404:414–417.
35. Caldas C, Myeong-Hee K, MacGregor A, Cain D, Aparicio S, et al. 1998. Isolation and characterisation of a Pufferfish MLL (mixed lineage leukaemia)-like gene (fMll) reveals evolutionary conservation in vertebrate genes related Drosophila tritorax. Oncogene 16:3233–3241.
36. Kasten M, Szerlong H, Erdjument-Bromage H, Tempst P, Werner M, et al. 2004. Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. EMBO J 23:1348–1359.
37. Paro R, Hogness DS. 1991. Polycomb protein shares a homologous domain with a heterochromatin-associated protein of Drosophila. Proc Natl Acad Sci USA 88:263–267.
38. Jones DO, Cowell IG, Singh PB. 2000. Mammalian chromodomain proteins: Their role in genome organisation and expression. Bioessays 22:124–137.
39. Eissenberg JC. 2001. Molecular biology of the chromodomain: an ancient chromatin module comes of age. Gene 275:19–29.
40. Ball LJ, Murzina NV, Broadhurst RW, Raine AR, Archer S, et al. 1997. Structure of the chromatin binding (chromo) domain from mouse modifier protein 1. EMBO J 16:2473–2481.
41. Nielsen PR, Nietlispach D, Mott HR, Callaghan J, Bannister A, et al. 2002. Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. Nature 416:103–107.
42. Jacobs SA, Khorasanizadeh S. 2002. Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science 295:2080–2083.
43. Reuter G, Spierer P. 1992. Position effect variegation and chromatin proteins. Bioessays 14:605–612.
44. Cowell IG, Austin CA. 1997. Self-association of chromo domain peptides. Biochim Biophys Acta 2:198–206.
45. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. 2001. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292:110–113.
46. Jacobs SA, Taverna SD, Zhang Y, Briggs SD, Li J, et al. 2001. Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. EMBO J 20:5232–5241.
47. Bouazone K, Mitterweger A, Langst G, Imhof A, Becker PB, et al. 2002. The dMi-2 chromodomains are DNA binding modules important for ATP-dependent nucleosome binding and mobilization properties. EMBO J 21:2430–2440.
48. Akhtar A, Zink D, Becker PB. 2000. Chromodomains are protein-RNA interaction modules. Nature 407:405–409.
49. Gu W, Szauter P, Lecchesi JC. 1998. Targeting of MOF, a putative histone acetyl transferase, to the X chromosome of Drosophila melanogaster. Dev Genet 22:56–64.
50. Kelley RL, Meller VH, Gordadze PR, Roman G, Davis RL, et al. 1999. Epigenetic spreading of the Drosophila dosage compensation complex from roX RNA genes into flanking chromatin. Cell 98:513–522.
51. Meller VH, Gordadze PR, Park Y, Chu X, Stuckenholz C, et al. 2000. Ordered assembly of the roX RNAs into MSL complexes on the dosage-compensated X chromosome in Drosophila. Curr Biol 10:136–143.
52. Maison C, Bailly D, Peters AH, Quivy JP, Roche D, et al. 2002. Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. Nat Genet 30:329–334.
53. Nielsen AL, Oulad-Abdelghani M, Ortiz JA, Remboutsika E, Chambon P, et al. 2001. Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. Mol Cell 7:729–739.
54. Bertram MJ, Berube NG, Hang-Swanson X, Ran Q, Leung JK, et al. 1999. Identification of a gene that reverses the immortal phenotype of a subset of cells and is a member of a novel family of transcription factors-like genes. Mol Cell Biol 19:1479–1485.
55. Bertam MJ, Pereira-Smith OM. 2001. Conservation of the MORF4 related gene family: identification of a new chromo domain subfamily and a novel protein motif. Gene 266:111–121.
56. Smith ER, Pannuti A, Gu W, Steurnagel A, Cook RG, et al. 2000. The drosophilae MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. Mol Cell Biol 20: 312–318.
57. Eisen A, Utley RT, Nourani A, Allard S, Schmidt P, et al. 2001. The yeast NuA4 and Drosophila MSL complexes contain homologous subunits important for transcriptional regulation. J Biol Chem 276:3484–3491.
58. Allard S, Utley RT, Savard J, Clarke A, Grant P, et al. 1999. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. EMBO J 18: 5108–5119.
59. Roth SY, Denu JM, Allis CD. 2001. Histone acetyltransferases. Annu Rev Biochem 70:81–120.
60. Lahn BT, Tang ZL, Zhou J, Barndt RJ, Parvinen M, et al. 2002. Previously uncharacterized histone acetyltransferases implicated in mammalian spermatogenesis. Proc Natl Acad Sci USA 99:8707–8712.
61. Kleiman SE, Yogev L, Hauser R, Botchan A, Bar-Shira Maymon B, et al. 2003. Members of the CDY family have different expression patterns: CDY1 transcripts have the best correlation with complete spermatogenesis. Hum Genet 113:486–492.
62. Ivanova AV, Bonaduce MJ, Ivanov SV, Klar SJS. 1998. The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. Nat Genet 19:192–195.
63. Ogas J, Kaufmann S, Henderson J, Somerville C. 1999. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. Proc Natl Acad Sci USA 96:13839–13844.
64. Kehle J, Beuchle D, treuheit S, Christen B, Kennison JA, et al. 1998. dMi-2, a hunchback-interacting protein that functions in Polycomb repression. Science 282:1897–1900.
65. Zelewsky TV, Palladino F, Brunschwig K, Tobler H, Hajnal A, et al. 2000. The C. elegant Mi-2 chromatin-remodelling proteins function in vulval cell fate determination. Development 127:5277–5284.
66. Seelig HP, Moosbrugger I, Ehrfed H, Fink T, Renz M, et al. 1995. The major dermatomyositis specific Mi-2 autoantigen is a presumed helicase involved in transcriptional activation. Arthritis Rheum 38:389–1399.
67. Ahringer J. 2000. NuRD and SIN3 histone deacetylase complexes in development. Trends Genet 16:351–356.
68. Woodage T, Basrai MA, Baxevanis AD, Hieter P, Collins FS. 1997. Characterization of the CHD family of proteins. Proc Natl Acad Sci USA 94:11472–11477.
69. Schuster EF, Stoeger RJ. 2002. CHD5 defines a new subfamily of chromodomain-SWI2/SNF2-like helicases. Mamm Genome 13:117–119.
70. Aasland R, Stewart AF, Gibson T. 1996. The SANT domain: a putative DNA-binding domain in the SWI_SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIB. Trends Biochem Sci 21:87–88.
71. Grüne T, Brzeski J, Eberharter A, Clapier CR, Corona DF, et al. 2003. Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. Mol Cell 12:449–460.
72. Ogata K, Morikawa S, Nakamura H, Sekikawa A, Inoue T, et al. 1994. Crystal structure of a specific DNA complex of the Myb DNA-binding domain with cooperative recognition helices. Cell 79:639–648.
73. Boyer LA, Langer MR, Crowley KA, Tan S, Denu JM, et al. 2002. Essential role for the SANT domain in the functioning of multiple chromatin remodeling enzymes. Mol Cell 4:935–942.
74. Boyer LA, Latek RR, Peterson CL. 2004. The SANT domain: a unique histone-tail-binding module? Nat Rev Mol Cell Biol 5:158–163.
75. Yu J, Li Y, Ishizuka T, Guenther MG, Lazar MA. 2003. A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. EMBO J 13:3403–3410.
76. You A, Tong JK, Grozinger CM, Schreiber SL. 2001. CoRest is an integral component of the CoREST human histone deacetylase complex. Proc Natl Acad Sci USA 98:1454–1458.
77. Fischle W, Dequiedt F, Fillion M, Hendzel MJ, Voelter W, et al. 2001. Human HDAC7 histone deacetylase activity is associated with HDAC3 in vivo. J Biol Chem 38:35826–35835.
78. Sterner DE, Wang X, Bloom MH, Simon GM, Berger SL. 2002. The SANT domain of Ada2 is required for normal acetylation of histones by the yeast SAGA complex. J Biol Chem 277:8178–8186.
79. Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, et al. 2002. MLL targets SET domain methyltransferase activity to Hox gene promoters. Mol Cell 5:1107–1117.

80. Platero JS, Harnett T, Eissenberg JC. 1995. Functional analysis of the chromo domain of HP1. EMBO J 14:3977–3986
81. Wang G, Ma A, Chow CM, Horsley D, Brown NR, et al. 2000. Conservation of heterochromatin protein 1 function. Mol Cell Biol 20:6970–6983.
82. Kanno T, Kanno Y, Siegel RM, Jang MK, Lenardo MJ, et al. 2004. Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. Mol Cell 1:33–43.
83. Cosma MP, Tanaka T, Nasmyth K. 1999. Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. Cell 97:299–311.
84. Ornaghi P, Ballario P, Lena AM, Gonzalez A, Filetici P. 1999. Bromodomain of Gcn5p interacts in vitro with specific residues in the N terminus of histone H4. J Mol Biol 1:1–7.
85. Volpe TA, Kidner C, Hall IM, Teng G, Grewall SIS, et al. 2002. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science 297:1833–1837.
86. Richards EJ, Elgin SC. 2002. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. Cell 108:489–500.
87. Kraulis PJ. 1991. MOLSCRIPT: A Program to Produce Both Detailed and Schematic Plots of Protein Structures. J Appl Cryst 24:946–950.
88. Shannon CE. 1948. A mathematical theory of communication. Bell System Tech J 27:379–423.
89. Valdar WS. 2002. Scoring residue conservation. Proteins 48:227–241.
90. Bateman A, Coin L, Durbin R, Finn RD, Hollich V, et al. 2004. The Pfam Protein Families Database. Nucleic Acids Res Database Issue 32:D138–D141.
91. Letunic I, Goodstadt L, Dickens NJ, Doerks T, Schultz J, et al. 2002. Recent improvements to the SMART domain-based sequence annotation resource. Nucleic Acids Res 30:242–244.