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THE BROMODOMAIN: A CHROMATIN BROWSER?

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1. ABSTRACT

Reversible modification of histone tails is a regulatory step in chromatin remodeling. The N-terminal tails of histones are signaling platforms that carry amino acid residues for post-translational modification and contribute to chromosomal higher order structure. These modifications are performed by a number of chromatin modulators such as histone (h) acetyltransferase, h-deacetylase, h-methyltransferase and h-kinase. Large numbers of these enzymes as well as other chromatin-associated proteins share the bromodomain, a signature protein motif. Structural studies reveal not only wide structural conservation of bromodomains but also envision a possible role of this domain in the recognition of specific modified residues in the histone tails. The widespread presence of bromodomains in leukemogenic and cancer genes has provided a fundamental tool for studies of the role of epigenetic and chromatin remodeling in malignant diseases.

2. INTRODUCTION

Since the first discovery of post-translational modification activities that regulate chromatin remodeling and gene expression, histone tails have set the stage in chromatin regulation as real platform for chromatin signaling (1,2). The exposed and disordered histone tails that protrude out of the core nucleosome are important for affecting chromatin structure (3), proper nucleosome assembly (4) and regulation of transcription (5). In addition, the solved 2.8 Angstrom crystal structure of the nucleosome core particle suggested a model in which the histone H4 tail induces a compacted higher order chromatin structure by interacting with H2A-H2B tetramer of the adjacent nucleosome (6). Furthermore, histone tails are required for chromatin remodeling by the SWI/SNF (7,8) and RSC complexes (9).

The discovery of *Tetrahymena* histone acetyltransferase-A and its yeast homolog Gcn5p, a member of the ADA and SAGA complexes (10,11), paved the way for the identification of a growing number of enzymes that modify the highly conserved histone tails (6,12). A structurally conserved GNAT superfamily includes hundreds of acetyltransferases (13), including P/CAF (14), p300/CBP (15,16), TAFII250 (17) and hGCN5 (18). In addition, there is an expanding number of signal transduction pathways (1) that activate histone H3 kinase (19) and transcription at specific chromosomal loci (20). Phosphorylation is often coupled to histone acetylation (21,22) and, interestingly, Gcn5p shows

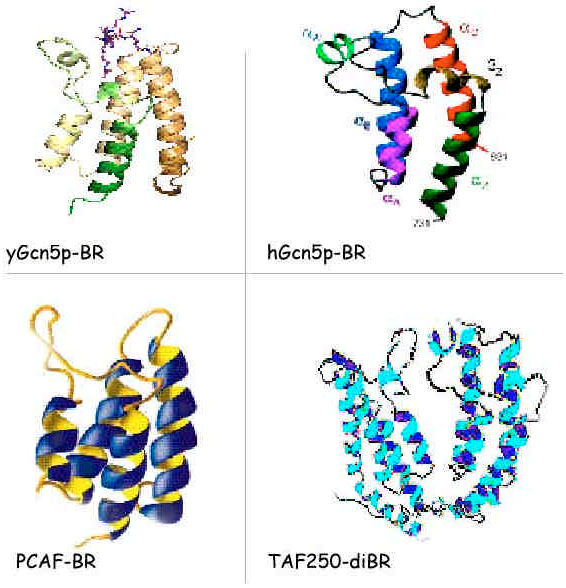

Figure 1. Bromodomain structure. Ribbon diagrams showing the bundle of four main alpha helices (A, Z, B and C) for all the already known bromodomain structures. In the case of yGCN5, the complex is shown with H4 N-acetyl lysine peptide positioned on the top.

increased HAT activity on H3 tails that have been phosphorylated at Ser-10 (21,23). Recently, a link between histone methylation and acetylation has been reported, based on the observation that a methyltransferase activity coimmunoprecipitates with CBP (24). At a genomic level, the mitotic condensation of chromosomes is linked to Ser-10 phosphorylation (25). Moreover, it has been proposed that localized or long-distance acetylation and deacetylation waves may account for locus-specific or generalized chromatin condensation and decondensation (26), which underlie each cell cycle round of cell duplication. A rapid and reversible turn over of acetylated and deacetylated nucleosomes would, in fact, guarantee the dynamism of chromatin structure at promoters to prevent a fixed, unresponsive chromatin state and ensure a ready transcriptional response (4).

In contrast, a closed, silenced chromatin state is induced by deacetylase complexes which may, in addition, recruit transcriptional repressors (27) or histone-methyltransferase activity (28) to further contribute to the silenced organization. Recruitment of silencing proteins, such Sir2 and 3, to H3 and H4 tails (29) and HP1 (heterochromatic protein) to H3 methyl-lysine 9 (30,31), can further reinforce the inactive conformation. Additional connections have been observed between phosphorylation and methylation. For example, the conserved SET domain in SUV39H1 protein shows a K-specific H3-methyltransferase activity that interferes with H3-phosphorylation and induces an abnormal chromosome condensation in the *suv39* mutant (28). Collectively, these and other data strengthen the idea of a histone code (1,2), composed of a combinatorial network of post-translational modifications, which are mutually interconnected and responsible for the recruitment of chromatin-associated proteins like activators, repressors and silencing proteins.

3. DISCUSSION

3.1. The Bromodomain

The recurring presence of the bromodomain signature module, first found in the *Drosophila* brahma protein (brm) (32), in almost all known histone acetyltransferases, in transcriptional coactivators and in components of chromatin-associated multiprotein complexes (33,34), was the motivating reason for consideration of this domain as a histone-interacting unit (35). Sequence and structural analysis indicate that bromodomains are highly conserved modules. Yet, the clear connection between different bromodomain-related functions awaits a comprehensive explanation. Its general definition as a "protein-protein interaction domain" (33) still reflects its pleiotropic function. Although it has been clearly established that bromodomains recognize acetyl-lysine of histone H4 (36,37,38,39), their role in contacting other tail modifications like phosphorylated and methylated residues has not yet been elucidated. Preliminary data on the interaction between the H3 N-terminus and the yGcn5p bromodomain (unpublished results) clearly suggest the existence of other possible complexes between bromodomains and a wide range of modifications.

The central importance of the bromodomain as chromatin targeting module (2) will be the focus of the last part of this review, starting with the observation of its presence in many different chromatin-associated proteins and gene fusions. The resultant transactivator fusion proteins are modular chimerae that are able to induce developmental switches (40) and malignant transformations (41).

3.1.1. The Bromodomain structure

The solution structure of the P/CAF (36) and human hGcn5p (39) bromodomains as well as the crystal structures for the TAFII250 dibromodomain (37) and yeast yGcn5p (38) reveal a highly conserved four-helix, left-twisted bundle with a pronounced hydrophobic cleft between the two conserved loops. We have combined the described three-dimensional structures (Figure 1) in order to show their striking similarity, which is in agreement with the highly conserved amino acid sequence and accounts for common function.

The interaction of the bromodomain with acetyl-lysine accounts for its role in acetylation-mediated transcriptional activation and nucleosome remodeling (42). However, it is still to be understood fully whether specific residues account for different functions among different bromodomains and if duplicated repeats exert different roles, such as in the case of dibromo-proteins like BDF1 (43,44) and TAFII250 (17), or poly-bromo proteins (45,46).

3.1.2. Sequence conservation and interacting residues

The bromodomain acetyl-lysine-binding pocket described in known structures is surrounded by two long

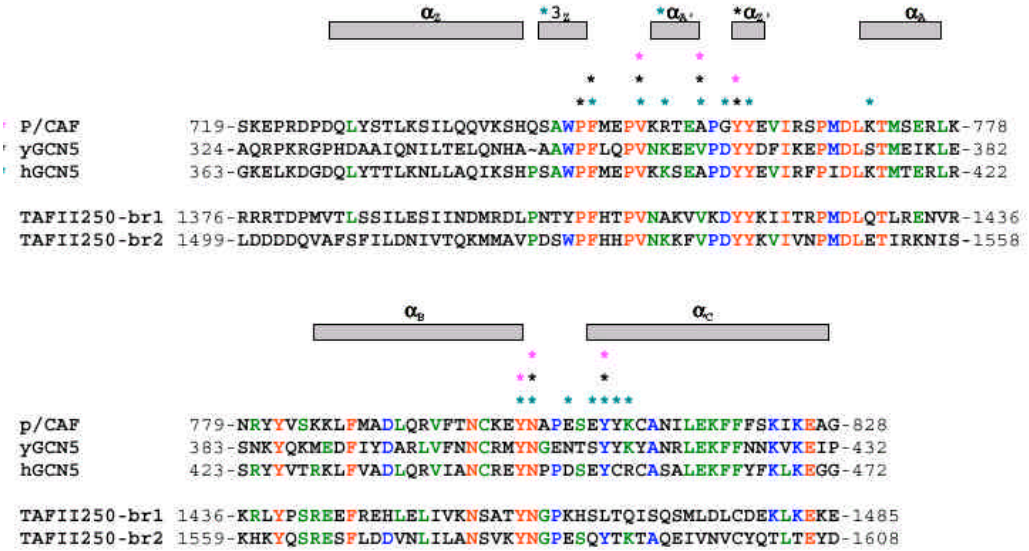

Figure 2. Conservation and secondary structure of the bromodomain. The secondary structure is depicted in the upper part of the Figure. In the multiple alignment, the asterisks correspond to the amino acid residues involved in H4 N-acetyl lysine interactions. The colors of the asterisks indicate the protein that makes the contact (red, P/CAF; black, yGCN5; and blue, hGCN5). The colors of the amino acid residues are: red for identity (5/5), blue for high conservation (4/5) and green for low conservation (3/5).

unstructured ZA and BC loops. Additional specific short helices are located within the ZA loop like alpha-3Z and alpha-A' in hGcn5p (39) and alpha-Z' in yGcn5p (38). The sequence alignment of bromodomains for which the three dimensional structure has been solved is shown in Figure 2. The residues that contact acetyl-lysine have been marked according to the literature (36,38,39). We have omitted to mark the interacting residues on TAFII250 bromodomains 1 and 2 because they were not stated in the original manuscript and because cooperativity between the two repeats may imply a more complex recognition mechanism. It is noteworthy that the common interacting amino acids, like valine in the ZA-loop, asparagine in the BC-loop and tyrosine at the N-terminus of helix alpha –C, are almost invariant. It is also clear that in almost all of the complexes analyzed, hydrophobic interacting residues are primarily restricted to the bromodomain loops. Conserved and interacting residues are highlighted in the alignment. It is also noteworthy that in the crystallization process of the yGcn5p bromodomain, an ordered crystal was obtained very quickly only after addition of acetylated H4-peptide, which suggests the existence of fluctuations in the unliganded bromodomain molecule. It will certainly be of interest to study the dynamic properties of the bromodomain in solution, in order to understand how its conformation accommodates histone modifications like phosphorylation or methylation and, conversely, if interactions with specific bromodomain modifications influence the dynamics of histone tail recognition.

3.1.3. H4 acetyl lysine binding site

Two highly conserved loops (ZA and BC loops) surround a profound hydrophobic cleft in the bromodomain structure to create an ideal binding pocket for contacting histone acetyl-lysine (Figure 1: yGcn5p-BR). In fact, crystal structures of the yGcn5p bromodomain (38) and the TAFII250 dibromodomain (37) show a strong preference for binding acetylated tails. In particular, TAFII250 binds the fully acetylated (K5, K8, K12 and K16) histone H4 tail with higher affinity. Cooperativity between the first and second bromodomain pockets has been proposed as a mechanism for contacting different acetyl-lysines.

The crystal structure of the Gcn5p bromodomain H4-AcK16 complex (38) demonstrates a preferential interaction of the bromodomain motif with AcK16. In addition, a secondary interacting site at position K+ 2 and K+ 3 has been described and accounts for an interaction with the unmodified H4 tail. This secondary, charged interaction site has been proposed to be important for the binding selectivity of different bromodomain proteins for targeted modifications. GST pull-down experiments identified the H4 N-terminal-interacting residues to be charged residues R19 and R23 (35). In addition, by a similar approach, the interacting residues of the unmodified histone H3 tail were defined, indicating once again the importance of charges in the binding reaction (unpublished results). Thus, consensus regions that flank the modified primary binding site might contribute to the interaction specificity of different bromodomain proteins and recognition of differently modified residues.

It is a still open question as to whether additional modifications like serine phosphorylation, or lysine and arginine methylation, influence or are influenced by the binding of the bromodomain. It is a future challenge to understand in greater detail how the bromodomain works. Is it a mere acetyl-lysine interaction motif or does it interact

with other modifications that carry a broader interaction specificity? To what extent does a cooperative interplay between primary and secondary interacting regions assist bromodomain interactions overall? Given the wide conservation of bromodomains in proteins with disparate functions, we are inclined to believe that bromodomains might interact with different targets through a common chromatin-anchoring function, ancillary to other catalytic motifs within the bromodomain protein.

3.2. Bromodomain mutant phenotypes

The *in vivo* functions of the bromodomain motif also remain an open question. The Gcn5p bromodomain was demonstrated to be dispensable for *in vitro* HAT function (10,47). It has been shown that the Gcn5p bromodomain is required for SAGA (Spt-Ada-Gcn5-acetyltransferase complex)-dependent HAT activity (48) but not for ADA (Adapter) function, which reveals a distinct effect on these two, partially overlapping, chromatin remodeling complexes. Similarly, a wide range of phenotypes has been linked to bromodomain deletion. In yeast, bromodomain deletion of Snf2/Swi2 (49), and in *Drosophila* of brm, shows no phenotype, whereas in humans, deletion of hbrm produces loss of nuclear localization and protein instability (50). Very recently, it was shown that hbrm protein is cleaved during apoptosis, which disrupts its nuclear association and interaction with other proteins (51). Among the members of RSC complex (Remodel the Structure of Chromatin), deletion of the Sth1 bromodomain induces a conditional lethal phenotype (52). In the dibromodomain proteins Rsc1 and Rsc2, deletion of the first repeat has no effect, whereas lack of the second bromodomain has a strong phenotypic inhibitory effect on cell growth (9). The observation that different bromodomains present in the same protein diverge more than bromodomains in different proteins suggests that the different repeats have different functions (34,53). The bromodomain of hGCN5 has also been reported to associate with the p70 subunit of the DNA-dependent protein kinase complex (PDK), a kinase complex that represses GCN5 HAT activity (54). It is therefore very difficult to find a common thread that connects the variety of described phenotypes. A leading criterion might be to envision the bromodomain as a histone tail-interacting domain, a “grappling hook” (55) that bridges acetylated or variably modified histone residues with chromatin modifiers and remodeling activities. The degree of divergence of bromodomains and the different association with other chromatin-related domains account for a wide spectrum of possible functions.

3.3. Chromatin remodeling

Gcn5 was first described as a transcriptional coactivator of yeast biosynthetic genes. Among these, HIS3 was reported to be one of the most affected by *gcn5* mutation (56). Along with the discovery of HAT function, GCN5 was described as a chromatin remodeler that is able to relocalize nucleosomes at HIS3 and PHO5 promoters in yeast (57,58), and requires the bromodomain for proper HIS3 transcriptional activation and chromatin remodeling (10). This evidence was reinforced by the finding that normal nucleosomal acetylation by the SAGA complex requires the Gcn5 bromodomain (48). Ordered recruitment of SWI/SNF and subsequent persistence of SAGA at the HO promoter has been proposed as an example of "epigenetic memory" that regulates the HO promoter at each cell cycle (59). Controversially, it was later suggested that the Gcn5p bromodomain is required for Swi2 recruitment and stabilization of the SWI/SNF complex at promoters followed by chromatin remodeling (42). More recently, it was demonstrated that, for SWI/SNF-dependent nucleosome disruption at promoters, retention of the complex requires the continued binding of the activator. Alternatively, a histone tail acetylation-mediated interaction is required (60), which invokes the bromodomain as a bridge for the ordered recruitment of different chromatin regulators on acetylated histone tails.

3.4. Bromodomain-associated motifs

Domain accretion in chromatin proteins has been proposed as an evolutionary mechanism for expansion of repeated chromatin-specific modules. Multipartite modular proteins evolved from a common, simple ancestor (61) to accomplish the chimeric association of different chromatin-related functions. Bromodomain-associated motifs have been extensively described (34); therefore we wish to present here only an overall summary. Helicase motifs are found in different transcriptional coactivators that cooperate with nuclear receptors, such as the estrogen and retinoic acid receptors (RAR) (62). Helicase motifs are also found in BRG1 (63), a human ortholog of yeast SWI2/SNF2 (7) and the cell cycle-related Sth1p (52), which has an associated ATPase activity.

The corepressor KAP-1 is composed of a PHD (plant homeodomain) and a bromodomain, and links the family of Kruppel-associated box (KRAB) zinc finger proteins (ZFP) to the histone deacetylase NuRD complex, and displays potent repression of transcription (64). The spatial conservation of PHD and bromodomain motifs is essential for optimal protein function, although the role of these domains is not unique because domains found in KAP-1 orthologs like TIF1-alpha (65) TIF1-beta (66) and TIF1-gamma (67) may display different roles. PHD domains are also found in other HATs like CBP and p300, *h*uASH1 and *D*rASH1 (68).

Kinase domains are present in TAFII250 and hCCG1 along with chromatin-linked domains like AT hooks, previously found in RSC protein complexes. The SET domain, first characterized as a suppressor of position effect variegation (PEV) in *Drosophila* (69) and later shown to exert a lysine-9-specific methyltransferase activity on the H3 tail, is found in the *Drosophila* Suv39h1 protein (28). The SET domain is also found associated with a bromodomain in hASH1 along with PHD and AT-hooks (68). Likewise, a truncated HMG box is found in a polybromo protein with five Br repeats and a BAH (bromo adjacent domain) (45).

3.5. Chromatin and diseases

The competing activity between acetylase and deacetylase determines gene expression and cell regulation. The specific recruitment of activator and chromatin

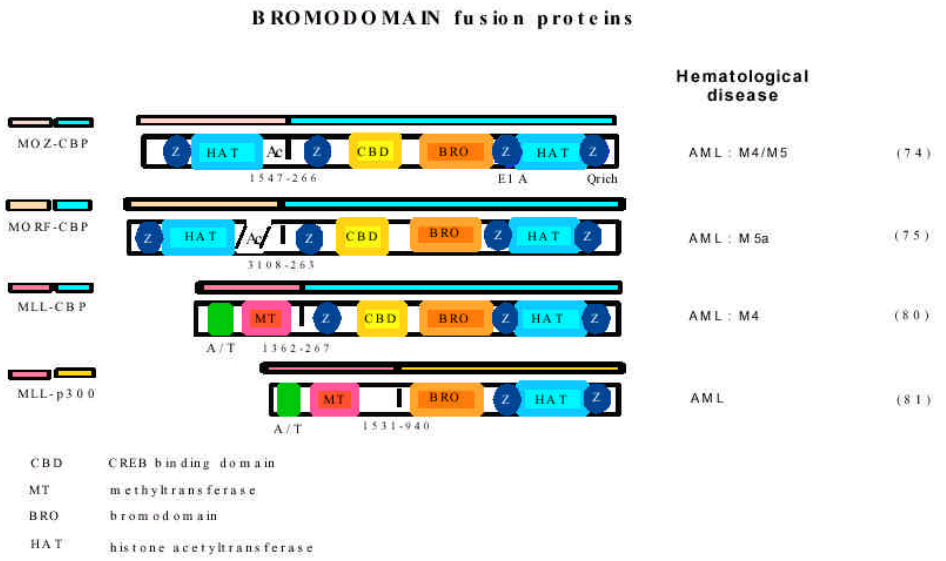

Figure 3. Bromodomain-fusions involved in malignancies. The proteins derived from MOZ, MORF or MLL fusions to CBP or p300 are depicted. Only functional domains inherent to the discussion are indicated (the scale of the single domains is arbitrary). The numbers under the breakpoint of the fusions indicate the amino acid residues in the original proteins.

remodelers at promoters so far studied contributes to a simplistic model of transcriptional regulation. However, chromatin structure and acetylation is responsible for an epigenetic regulation at a higher scale throughout the genome. The paradigm of local chromatin remodeling is the gene-specific recruitment of modification activities like HAT by activators (70) which, if perturbed, may induce a leukemogenic state. In the case of acute promyelocytic leukemia (APL), RAR binding of the ligand (retinoic acid) induces conformational changes, release of the repressing NCoR/Sin3/HDAC complex (Nuclear Receptor Co-Repressor/Histone Deacetylase Complex) and subsequent interaction with coactivator complexes that carry HAT activities (71,72). The novel association with HAT reverses the repressive effect of the deacetylase complexes and induces modification of nucleosomal structure and chromatin remodeling (41). Similarly, exchanged recruitment of coactivator for a corepressor is the mechanism by which the oncoprotein AML1-ETO alters gene expression and accounts for over 10% of acute myeloid leukemia (AML) (41). In the case of MLL–CBP-induced leukemogenic transformation, a gain of function is obtained by direct recruitment of acetyltransferase activity at target genes. The association of the tumor suppressor BRCA1, which has been linked to predisposition to breast and ovarian cancer, with the SWI/SNF complex reveals a direct function of BRCA1 in the modulation of chromatin structure (73). The extent to which localized modification of nucleosomal acetylation induces chromatin reorganization in erythroid-specific genes might represent a future challenge for investigation.

3.5.1. Fusion proteins

Alteration of chromatin structure and acetylation often gives rise to malignancies. In fact, many chromosomal translocations that lead to leukemia generate chimeric proteins that contain acetyltransferase domains (41). Here we focus on the chimeric proteins that contain HAT and bromodomain motifs and that are found in pathogenesis. It is significant that most of the fusions contain CBP with its HAT and bromodomain portion. CBP and p300 have been defined as tumor suppressor proteins that are essential in cellular differentiation as well as in human development (71,72). The described fusions are schematized in Figure 3. The first chromosomal translocation to be characterized was t(8;16) (p11;p13), in which MOZ (monocytic leukemia zinc finger protein) is fused to the CBP amino-terminus in the M4/M5 subtype of AML (74). In MOZ-CBP, the Zn-finger and HAT domain of MOZ are fused to a fragment of CBP that contains the CREB and E1A binding domains as well as a bromodomain and HAT. The resultant protein gives rise to two newly associated HAT domains and one bromodomain. The MORF-CBP chimera observed in chromosomal translocation t(10;16)(q22;p13) is found in childhood AML M5a (75). MORF (monocytic leukemia zinc finger protein-related factor) displays significant sequence similarity to MOZ. It has an intrinsic histone acetyltransferase activity, a strong transcriptional repression domain at its N-terminus and a highly potent activation domain at its C-terminus (76). The HAT and transcriptional repression domains of MORF are fused to CBP, which extends from the RAR binding site to its C-terminal Glu-rich domain. Two HAT domains are therefore associated in MORF-CBP. CBP is once more involved in the translocation t(11;16)(q23;p13) that occurs in *de novo* acute myelomonocytic leukemia (AML-M4) and rarely in treatment-related myeloid leukemia (77,78). In this case, CBP is fused to the MLL gene (mixed lineage leukemia), also called HRX, ALL-1 and Htrx (79). The chimeric protein produced contains the AT-hooks and the methyltransferase homology region of MLL associated with CBP regions found in other fusions described elsewhere (80). Less common translocations involve the fusion of MLL and p300, in the translocation t(11;22)(q23;q13) that occurs in acute myeloid leukemia

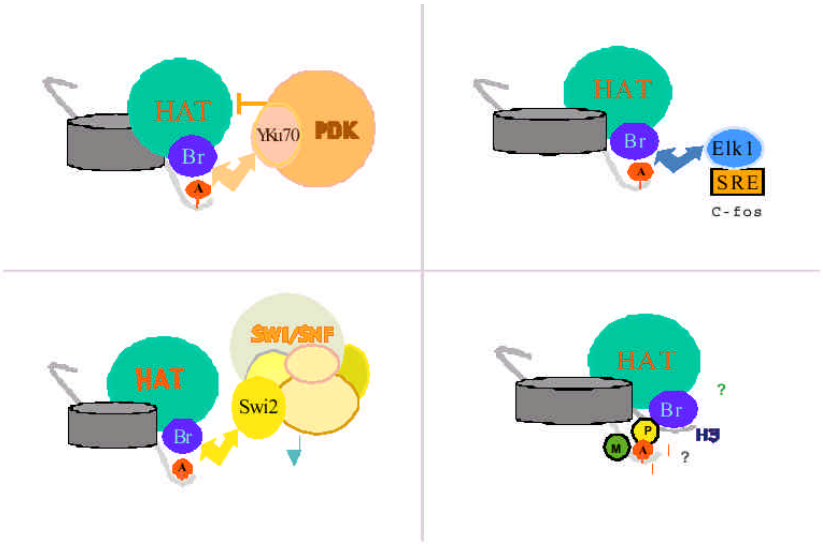

Figure 4. Bromodomain interactions. Interactions with different subunits of multiprotein complexes are shown, starting from the uppermost left and proceeding in a clockwise direction: the kinase yKu 70 belonging to the DNA-dependent protein kinase complex (PDK); the transcription factor Elk1, a member of the Ternary Complex that activates the c-fos promoter; hypothetical interactions of a bromodomain with different modifications of histone H3; and Swi2, the subunit of the ATPase-dependent remodeling complex SWI/SNF.

(81) and carries the p300 portion that contains the bromodomain, Zn-finger and HAT domains.

3.5.2. Rearranged coactivators and leukemia

Treatment-related myelodysplastic syndrome (t-MDS) or acute leukemia often involve translocation of the MLL gene at chromosomal location 11q23 (78). MLL is involved in translocation with many different partners (82). In a t(11;16) translocation, the created gene fusion MLL–CBP induces a leukemic phenotype *in vivo* (80), whereas in t(11;22,q23;q13), a fusion with p300 induces AML (81). MLL is homologous to the *Drosophila* trithorax (trx), which controls Bithorax and Antennapedia homeotic gene expression (83). Genetic evidence shows that the bi-directional homeotic transformation described in the *Mll* null mouse embryo (84) is similar to *trx* transformants in *Drosophila* (85). The etiology of MLL-CBP is thought to be related to a modification of chromatin remodeling, a hypothesis that is reinforced by the structural organization and the oncogenic property of the fusion. It has in fact been demonstrated that the full oncogenicity MLL-CBP is retained only if both the HAT domain and the bromodomain of CBP are present in the transforming fusion gene (80). The HAT domain alone showed catalytic but not transforming activity.

3.5.3. Cancer and apoptosis

In several cases, screens for new genes related to human malignancies have identified bromodomain proteins. The expression of a testis-restricted member of the RING3 family has recently been observed in lung cancer tissue (86). BRL, another gene that is related to AF10 and AF17 leukemia genes, was reported to have a testis-specific expression (87). Similarly, the deletion of p300 exon 15-18 in the SiHa cervical carcinoma cell line, which causes loss of the bromodomain by frame shift mutation, impaired p21 promoter activity in a transient reporter assay (88), which suggests a role for the p300 bromodomain in tumor suppression. In addition, an interesting result comes from a study of hbrm regulation related to hematopoietic cell differentiation. It was shown that in NB4 leukemic cells, cathepsin G cleaves and degrades hbrm upon induction of apoptosis by UV irradiation or chemotherapeutic agents (51). The presence of hbrm could provide some degree of resistance to UV radiation-induced apoptosis suggested by the higher rate of apoptosis after irradiation in hbrm knockout mice. In leukemic cells, degradation of hbrm might disrupt the hbrm-repressor complex (89) and interfere with G1 specific growth-arrest (90) and cell cycle regulation.

4. PERSPECTIVE

Since the first alignment of bromodomain-containing proteins (32), the repeated observation of this cryptic motif in many organisms has stimulated interest in the search for a function. As we have reported previously, the multiple functions and the pleiotropic actions of the bromodomain are elusive and do not support a simple model. In an effort to provide a possible interpretation for its role in protein-protein interaction, we have schematized in a panel (Figure 4) the different interactions exerted with different complexes.

The bromodomain interaction with the yKu70 subunit of the DNA-dependent protein kinase complex has been proposed to be a possible mechanism for the inhibition of HAT activity (54). This effect might be thought of as a feedback regulatory mechanism after the interaction with acetylated histone tails. Another important

contribution to the field of bromodomain interactions came from the recent finding that the CBP bromodomain recruits the Ternary Complex factor Elk-1 to the serum responsive element (SRE) of the c-fos (91) promoter. Is still unclear whether in this case the acetylated chromatin is required for Elk1 recruitment or how the overall chromatin structure influences this complex. Moreover, the Gcn5p bromodomain has been shown to play an important role in recruitment of Swi2 and subsequent SWI/SNF-dependent chromatin remodeling (42,60). However, it is controversial whether time-ordered entry of SAGA and SWI/SNF complexes is a general mechanism, because a reversed order of complex recruitment has been reported at the HO promoter (59).

Last but not least, we address the function of the bromodomain as a histone H3 and H4-interacting domain. Three-dimensional structures have highlighted the binding of the bromodomain to the acetylated H4 tail (36,37,38,39). In our opinion, besides interaction with acetyl-lysine, it remains to be established how and if other histone modifications or combination thereof may regulate the bromodomain interaction at specific loci (1). Collectively, these data support the notion of a bromodomain as a multifunctional protein motif that is able to interact with members of multiprotein complexes that are involved in different pathways. Overall, our leading and preferred idea is that a key step in chromatin remodeling is the interaction of bromodomains with histones; recognition of the histone code is its putative role.

The contribution of bromodomains to oncogenic fusion genes described above strongly argues for the involvement of chromatin regulation in human hematological disorders. It remains to be assessed whether direct interaction with histone tails or recruitment of specific factors like yKu70 and Elk-1 are the crucial steps in oncogenesis. An important issue is that, in any case, a so-called chromatin therapy might be thought of as a new therapeutic field.

We believe that further work on macromolecular structure will uncover other important features of the structural interaction between bromodomains and the nucleosome tails. In the near future, analysis of bromodomain point mutants along with a more careful study of the associated phenotypes will add to our comprehension of the bromodomain-related chromatin network.

5. ACKNOWLEDGMENT

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6. REFERENCES

1. Cheung, P., Allis, C.D., Sassone-Corsi, P.: Signaling to chromatin through histone modifications. *Cell* 103, 263-71 (2000)

2. Strahl, B.D., and Allis, C.D.: The language of covalent histone modifications. *Nature* 403, 41-5 (2000)

3. Fisher-Adams, G., Grunstein, M.: Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the GAL1 promoter. *EMBO J.* 14,1468-77 (1995)

4. Wu, J., Grunstein, M.: 25 years after the nucleosome model: chromatin modifications. *Trends Biochem Sci* 12,619-23 (2000)

5. Hansen, J.C., Tse, C., and Wolffe, A.P.: Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry* 37, 17637-41 (1998)

6. Luger, K., Richmond, T.J.: The histone tails of the nucleosome. *Curr Opin Genet Dev* 8,140-6 (1998)

7. Cote, J., Quinn, J., Workman, J.L., Peterson, C.L.: Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* 265, 53-60 (1994)

8. Pollard, K.J., Peterson, C.L.: Chromatin remodeling: a marriage between two families? *Bioessays* 9, 771-80 (1998)

9. Cairns, B.R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R.D., Winston, F.: Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH and bromodomains. *Mol Cell* 4, 715-23 (1999)

10. Marcus, G.A., Silverman, N., Berger, SL., Horiuchi, J., Guarente, L.: Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. *EMBO J* 13, 4807-15 (1994)

11. Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., Allis, C.D.: Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* 84, 843-51 (1996)

12. Kornberg, RD., Lorch, Y.: Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* 98, 285-94 (1999)

13. Dyda, F., Klein, D.C., Hickman, A.B.: GCN5-related N-acetyltransferases: a structural overview. *Ann Rev Biophys Biomol Struct* 29, 81-103 (2000)

14. Yang, X.J., Ogryzko, V.V., Nishikawa, J., Howard, B.H., and Nakatani, Y.A.: p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* 382, 319-324 (1996)

15. Bannister, A.J., and Kouzarides, T.: The CBP co-activator is a histone acetyltransferase. *Nature* 384, 641-43 (1996)

16. Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y.: The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* 87, 953-59 (1996)

17. Mizzen, C.A., Yang, X.Y., Kokubo, T., Brownell, J.A., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., Nakatani, Y., and Allis, C.D.: The TAF II 250 subunit of TFIID has histone acetyltransferase activity. *Cell* 87, 1261-70 (1996.)

18. Candau, R., Moore, P.A., Wang, L., Barlev, N., Ying, C.Y., Rosen, C.A., Berger, SL.: Identification of human proteins functionally conserved with the yeast putative adaptors ADA2 and GCN5. *Mol Cell Biol.* 2, 593-602 (1996)

19. Hsu, J.Y., Sun, Z.W., Li, X., Reuben, M., Tatchell, K., Bishop, D.K., Grushcow, J.M., Brame, C.J., Caldwell, J.A., Hunt, D.F., Lin, R., Smith, M.M., Allis, C.D.: Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding and nematodes. *Cell* 102, 279-91 (2000)

20. Nowak, S.J., Corces, V.G.: Phosphorylation of histone H3 correlates with transcriptionally active loci. *Genes Dev* 14, 3003-13 (2000)

21. Cheung, P., Tanner, K.G., Cheung, W.L., Sassone-Corsi, P., Denu, J.M., Allis, C.D.: Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol Cell* 6, 905-15 (2000)

22. Clayton, A.L., Rose, S., Barratt, M.J., Mahadevan, L.C.: Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. *EMBO J* 19, 3714-26 (2000)

23. Lo, W.S., Trievel, R.C., Rojas, J.R., Duggan, L., Hsu, J.Y., Allis, C.D., Marmorstein, R., Berger, S.L.: Phosphorylation of serine 10 in histone H3 is functionally linked *in vitro* and in vivo to Gcn5-mediated acetylation at lysine 14. Mol Cell 6, 917-26 (2000)

24. Vandel, L. and Trouche, D.: Physical association between the histone acetyltransferase CBP and histone methyltransferase. *EMBO Rep* 2, 21-6 (2001)

25. Wei, Y., Yu, L., Bowen, J., Gorovsky, M.A., Allis, C.D.: Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell* 97, 99-109 (1999)

26. Vogelauer, M., Wu, J., Suka, N., Grunstei, M.: Global histone acetylation and deacetylation in yeast. *Nature* 408, 495-8 (2000)

27. Ng, H.H., Bird, A.: Histone deacetylases: silencers for hire. *Trends Biochem Sci* 3,121-6 (2000)28. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., Jenuwein, T.: Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593-9 (2000)

29. Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S.M., and Grunstein, M.: Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. *Cell* 80, 583-92 (1995)

30. Lachner, M., O'Carrol, D., Rea, S., Mechtler, K., and Jenuwein, T.: Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410,116-20 (2001)

31. Bannister, A.J., Zegerman, P., Partridge, JF., Miska, E.A., Thomas, J.O., Allshire, R.C., and Kouzarides, T.: Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromodomain. *Nature* 410,120-4 (2001)

32. Tamkun, J.W., Deuring, R., Scott, M.P., Kissinger, M., Pattatucci, A.M., Kaufman, T.C., and Kennison, J.A.: brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* 68, 561-72 (1992)

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

34. Jeanmougin, F., Wurtz, J.M., Le Douarin, B., Chambon, P., Losson, R.: The bromodomain revisited. *Trends Biochem Sci* 22, 151-3 (1997)

35. Ornaghi, P., Ballario, P., Lena, A.M., Gonzàlez, A., and Filetici, P.: The bromodomain of Gcn5p interacts *in vitro* with specific residues in the N-Terminus of histone H4. *J Mol Boil* 287,1-7 (1999)

36. Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., Zhou, M.M.: Structure and ligand of a histone acetyltransferase bromodomain. *Nature* 399, 491-6 (1999)

37. Jacobson, R.H., Ladurner, A.G., King, D.S., Tjian, R.: Structure and function of a human TAFII250 double bromodomain module. *Science* 288, 1422-5 (2000)

38. Owen, D.J., Ornaghi, P., Yang, J.C., Lowe, N., Evans, P.R., Ballario, P., Neuhaus, D., Filetici, P., Travers, A.A.: The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase Gcn5p. *EMBO J* 19, 6141-9 (2000)

39. Hudson, B.P., Martinez-Yamout, M.A., Dyson, H.J., Wright, P.E.: Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. *J Mol Biol.* 304, 355-70 (2000)

40. Mannervik, M., Nibu, Y., Zhang, H., and Levine, M.: Transcriptional coregulators in development. *Science* 284, 606-9 (1999)

41. Redner, R.L., Wang, J., and Johnson, M.L.: Chromatin remodeling and Leukemia: new therapeutic paradigms. *Blood* 94, 417-28 (1999)

42. Syntichaki, P., Topalidou, I., and Thireos, G.: The Gcn5p bromodomain co-ordinates nucleosome remodeling. *Nature* 404, 414-17 (2000)

43. Chua, P., Roeder, G.S.: Bdf1, a yeast chromosomal protein required for sporulation. *Mol Cell Biol.* 15, 3685-96 (1995)

44. Matangkasombut, O., Buratowski, R.M., Swilling, N.W., Buratowski, S.: Bromodomain factor 1 corresponds to a missing piece of yeast TFIID. *Genes Dev* 8, 951-62 (2000)

45. Nicolas, RH., Goodwin, GH.: Molecular cloning of polybromo, a nuclear protein containing multiple domains including five bromodomains, a truncated HMG-box, and two repeats of a novel domain. *Gene* 175, 233-40 (1996)

46. Geisberg, J.V., Chen, J.L., Ricciardi, R.P.: Subregions of the adenovirus E1A transactivation domain target multiple components of the TFIID. *Mol Cell Biol.* 11, 6283-90 (1995)

47. Candau, R., Zhou, J.X., Allis, C.D., and Berger, S.L.: Histone acetyltransferase activity and interaction with ADA2 are critical for Gcn5p function *in vitr*o. *EMBO J* 16, 555-65 (1997)

48. Sterner D.E, Grant P.A, Roberts S.M, Duggan L.J, Belotserkovskaya R, Pacella L.A, Winston F, Workman J.L, Berger S.L Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. *Mol Cell Biol.* 1, 86-98 (1999)

49. Laurent, B.C., Treich, I., Carlson, M.: The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. *Genes Dev* 7, 583-91 (1993)

50. Muchardt, C., and Yaniv, M.: A human homologue of Saccharomyces cerevisiae SNF2/SWI2 and Drosophila brm genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J* 12, 4279-90 (1993)

51. Biggs, J.R., Yang, J., Gullberg, U., Muchardt, C., Yaniv, M., Kraft, AS.: The human brm protein is cleaved during apoptosis: The role of cathepsin. *Proc Natl. Acad. Sci* 98, 3814-9 (2001)

52. Du, J., Nasir, I., Benton, B.K., Kladde, M.P., Laurent, B.C.: Sth1p, a Saccharomyces cerevisiae Snf2p/Swi2p homolog, is an essential ATPase in RSC and differs Snf/Swi in its interactions with histones and chromatin-associated. *Genetics* 150, 987-1005 (1998)

53. Winston, F., and Allis, C.D.: The bromodomain: a chromatin-targeting module? *Nature* 6, 601-4 (1999)

54. Barlev, N.A., Poltoratsky, V., Owen-Hughes, T., Ying, C., Liu, L., Workman, J.L., Berger, S.L.: Repression of GCN5 histone acetyltransferase activity via bromodomain-mediated binding and phosphorylation by the Ku-DNA-dependent protein kinase complex. *Mol Cell Biol.* 18, 1349-58 (1998)

55. Travers, A.: How to put a HAT on the histones. *Cur Biol.* 9, 23-25 (1999)

56. Georgakopoulos, T., Thireos, G.: Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J* 11, 4145-52 (1992)

57. Filetici, P., Aranda, C., Gonzalez, A., Ballario, P.: GCN5, a yeast transcriptional coactivator, induces chromatin reconfiguration of HIS3 promoter in vivo. *Biochem Biophys Res Commun* 242, 84-7 (1998)

58. Gregory, P.D., Schmid, A., Zavari, M., Lui, L., Berger, S.L., Horz, W.: Absence of Gcn5 HAT activity defines a novel state in the opening of chromatin at the PHO5 promoter in yeast. *Mol Cell* 1,1495-505 (1998)

59. Cosma, M.P., Tanaka, T., Nasmyth, K.: Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle and developmentally regulated promoter. *Cell* 97, 299-311 (1999)

60. Hassan, AH., Neely, KE., and Workman, JL.: Histone Acetyltransferase complexes stabilize SWI/SNF binding to promoter nucleosomes. *Cell* 104, 817-27 (2001)

61. International Human Genome Sequencing Consortium.: Initial sequencing and analysis of the human genome. *Nature* 409, 860-921 (2001)

62. Chiba, H., Muramatsu, M., Nomoto, A., Kato, H.: Two human homologues of Saccharomyces cerevisiae SWI2/SNF2 and Drosophila brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic Acids Res* 10,1815-20 (1994)

63. Randazzo, F.M., Khavari, P., Crabtree, G., Tamkun, J., Rossant, J.: brg1: a putative murine homologue of the Drosophila brahma gene, a homeotic gene regulator. *Dev Biol.* 161, 229-42 (1994)

64. Schultz, D.C., Friedman, J.R., and Rauscher III, F.J.: Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha .subunit of NuRD. *Genes and Dev* 15, 428-43 (2001)

65. Le Douarin, B., You, J., Nielsen, A.L., Chambon, P., Losson, R.: TIF1alpha: a possible link between KRAB zinc finger proteins and nuclear receptors. *J Steroid Biochem Mol Biol.* 65, 43-50 (1998)

66. Chang, C.J., Chen, YL., Lee, S.C.: Coactivator TIF1beta interacts with transcription factor C/EBP beta and glucocorticoid receptor to induce alpha1-acidglycoprotein gene expression. *Mol Cell Biol.* 10, 5880-7 (1998)

67. Venturini, L., You, J., Stadler, M., Galien, R., Lallemand, V., Koken, M.H., Mattei, M.G., Ganser, A.,Chambon, P., Losson, R., de The, H.: TIF1gamma, a novel member of the transcriptional intermediary factor 1 famil*y. Oncogene* 18,1209-17 (1999)

68. Nakamura, T., Blechman, J., Tada, S., Rozovskaia, T., Itoyama, T., Bullrich, F., Mazo, A., Croce, C.M., Geiger, B., Canaani, E.: huASH1 protein, a putative transcription factor encoded by a human homologue of the Drosophila ash1 gene, localizes to both nuclei and cell-cell tight junctions. *Proc Natl. Acad. Sci* 13, 7284-9 (2000)

69. Melcher, M., Schmid, M., Aagaard, L., Selenko, P., Laible, G., Jenuwein, T.: Structure-function analysis of SUV39H1 reveals a dominant role in heterochromatin organization, chromosome segregation, and mitotic progression. *Mol Cell Biol.* 10,3728-41 (2000)

70. Utley, R.T., Ikeda, K., Grant, PA., Cote, J., Steger, D.J., Eberharter, A., John, S., Workman, J.L.: Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. *Nature* 394, 498-502 (1998)

71. Goodman, R.H., and Smolik, S.: CBP/p300 in cell growth, transformation and development. *Genes Dev* 14, 1553-77 (2000)

72. Giles, R.H., Peters, D.J.M. and Breuning, MH.: Cojunction dysfunction: CBP/p300 in human disease *TIG* 14, 178-83 (1998)

73. Bochar, D.A., Wang, L., Beniya, H., Kinev, A., Xue, Y., Lane, W.S., Wang, W., Kashanchi, F., Shiekhattar, R.: BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell* 102, 257-65 (2000)

74. Borrow, J., Stanton, V.P., Andresen M., Becher, R., Behm, F.G., Chaganti, R.S.K., Civin, C.I., Disteche, C., Dube, I., Frischauf, A.M., Horsman, D., Mitelman, F., Volinia, S., Watmore, A.E., Housman, D.E.: The translocation t(8;16)(p11;p13) of acute myeloid leukemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat Genet* 14, 33-41 (1996)

75. Panagopoulos, I., Fioretos, T., Isaksson, M., Samuelsson, U., Billstrom, R., Strombeck, B., Mitelman, F., Johansson, B.: Fusion of the MORF and CBP genes in acute myeloid leukemia with the t(10;16)(q22;p13) *Hum Mol Genet* 15, 395-404 (2001)

76. Champagne, N., Bertos, NR., Pelletier, N., Wang, AH., Vezmar, M., Yang, Y., Heng, H.H., and Yang, X.: Identification of human histone acetyltransferase related to monocytic leukemia zing finger protei*n. J Biol. Chem.* 274, 28528-36 (1999)

77. Sobulo, O.M., Borrow, J., Tomek, R., Reshmi, S., Harden, A., Schlegelberger, B., Housman, D., Dogget, N.A., Rowley, J.D.: MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3) *Proc. Natl. Acad. Sci* 94, 8732-37 (1997)

78. Rowley, J.D., Reshmi, S., Sobulo,O., Musvee, T., Anastasi, J., Raimondi, S., Schneider, N.R., Barredo, J.C., Canti, E.S. Schlegelberger, B., Behm, F., Dogget, N.A., Borrow, J., and Zeleznik-Le, N.: All patients with the t(11;16)(q23;p13,3) that involves MLL and CBP have treatment-related hematologic disorders. *Blood* 90,535-41 (1997)

79. Djabali, M., Selleri, L., Parry, P., Bower, M., Young, B.D., Evans, GA.: A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. *Nat Genet* 2,113-8 (1992)

80. Lavau, C., Du, C., Thirman, M., and Zeleznik-Le, N.: Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia. *EMBO J* 19, 4655-64 (2000)

81. Ida, K., Kitabayashi, I., Taki, T., Taniwaki, M., Noro,K., Yamamoto, M., Ohki, M., and Hayashi, Y.: Adenoviral E1A-associated protein p300 is involved in acute myeloid leukemia with t(11;22) (q23;q13) *Blood* 90, 4699-4704 (1997)

82. Bernard, O.A., and Berger, R.,: Molecular basis of 11q23 rearrangements in hematopoietic malignant proliferations. *Genes and Chromosomes Cancer* 13, 75-85 (1995)

83. Kennison, J.A.: The Polycomb and trithorax group proteins of Drosophila: trans-regulators of homeotic gene function. *Ann. Rev Genet* 29,289-303 (1995)

84. Hanson, R.D., Hess, J.L., Yu B.D., Ernest, P., van Lohuizen, M., Berns, A., van der Lugt, N.M., Shashikant, C.S., Ruddle, F.H., Seto, M., Korsmeyer, S.J.: Mammalian Trithorax and polycomb-group homologues are antagonistic regulators of homeotic development. *Proc Natl. Acad. Sci U S* A. 96, 14372-7 (1999)

85. Yu, B.D., Hess, J.L., Horning, S.E., and Korsmeyer, S.J.: Altered Hox expression and segmental identity in *Mll*-mutant mice. *Nature* 378, 505-8 (1995)

86. Scanlan, M.J., Altorki, N.K., Gure, A.O., Williamson, B., Jungbluth, A., Chen, Y.T., Old, L.J.: Expression of cancer-testis antigens in lung cancer: definition of bromodomain testis-specific gene (BRDT) as a new CT gene, CT9. *Cancer Lett* 150, 155-64 (2000)

87. McCullagh, P., Chaplin, T., Meerabux, J., Grenzelias, D., Lillington, D., Poulsom, R. Gregorini,A., Saba, V., Yong, B.D.: The cloning, mapping and expression of a novel gene, BRL, related to the AF10 leukaemia gene. *Oncogene* 18, 7442-52 (1999)

88. Ohshima, T., Suganuma, T., Ikeda, M. A novel mutation lacking the bromodomain of the transcriptional coactivator p300 in the SiHa cervical carcinoma cell line. *Biochem Biophys. Res. Commun.* 281, 569-75 (2001)

89. Zhang, H.S., Gavin, M., Dahiya, A., Postigo, A.A., Ma, D., Luo, RX., Harbour, J.W., Dean, D.C.: Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/ and Rb-hSWI/SNF. *Cell* 101, 79-89 (2000)

90. Shanahan, F., Seghezzi, W., Parry, D., Mahony, D., Lees, E.: Cyclin E associates with BAF155 and BRG1, components of the mammalian SWI-SNF complex, and alters ability of BRG1 to induce growth arrest. *Mol Cell Biol.* 2,1460-9 (1999)

91. Nissen, L.J., Gelly, J.C., Hipskind, R.A.: Induction-independent recruitment of CREB-binding protein to the c-fos Serum Response Element through interactions between the Bromodomain and Elk-1. *J Biol. Chem.* 276, 5213-21 (2001)

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