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Selective targeting of histone methylation

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Histones are post-translationally modified by multiple histone-modifying enzymes, which in turn influences gene expression. Much of the work in the field to date has focused on genetic, biochemical and structural characterization of these enzymes. The most recent genome-wide methods provide insights into specific recruitment of histone-modifying enzymes in vivo and, therefore, onto mechanisms of establishing a differential expression pattern. Here we focus on the recruitment mechanisms of the enzymes involved in the placement of two contrasting histone marks, histone H3 lysine 4 (H3K4) methylation and histone H3 lysine 27 (H3K27) methylation. We describe distribution of their binding sites and show that recruitment of different histone-modifying proteins can be coordinated, opposed or alternating. Specifically, genomic sites of the H3K4 histone demethylase KDM5A become accessible to its homolog KDM5B in cells with a lowered KDM5A level. The currently available data on recruitment of H3K4/H3K27 modifying enzymes suggests that the formed protein complexes are targeted in a sequential and temporal manner, but that additional, still unknown, interactions contribute to targeting specificity.

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Introduction

The silenced and activated gene expression pattern is determined by transcription factors, which bind to DNA or chromatin. While chromatin organization can be retained, reassuring cellular identity and genome integrity, it is not as stable an edifice as DNA. In response to the activity of bound transcription factors, chromatin can undergo changes resulting in differential gene expression. This ensures that gene expression can be rapidly modified during normal biological processes such as DNA replication, recombination, response to DNA damage and other types of stress, cell cycle progression and differentiation. The basic unit of chromatin is the nucleosome representing ~147 base pairs of DNA wrapped around a histone core that consists of two copies each of histones H2A, H2B, H3 and H4. Each of the histones can be covalently modified in a number of ways, including methylation, acetylation, phosphorylation and ubiquitination.[1] Histone modification was originally predicted to exist in an almost unlimited number of possible combinations that would determine distinct transcriptional outcomes, known as a histone code hypothesis.[2] However, further studies showed that the transcription level is determined by a relatively small number of histone modifications[3] including methylation at H3K4. This phenomenon is common across different cell types in human as well as across different species.[4] Methylation at H3K4 is associated with promoter regions of actively transcribed genes. Methylation at lysine residues can occur in the form of mono, di- or trimethylation. The trimethylation of H3K4 (H3K4me3) marks the transcription start sites of active genes,[5-7] whereas monomethylation of H3K4 (H3K4me1) marks the enhancers of tissue-specific and stimulus-responsive genes.[8]

Methylation depends on two antagonizing groups of enzymes, "writers" and "erasers," which can install and remove histone marks, respectively.[9] Di- and

trimethylation at H3K4 is performed by the SET1 and mixed lineage leukemia (MLL) family of histone methyltransferases (HMTs). The "erasing" of K4 methylation is accomplished by the jumonji AT-rich interactive domain 1 (JARID1/KDM5) family of histone demethylases (HDMs) to which KDM5A and KDM5B belong.

Aside from its function as a binding site for proteins with catalytic activity, the N-trimethylated form of lysine 4 of histone H3 is known to be a docking site for a variety of "reader" proteins through their plant homeobox domains (PHDs), chromodomains, WD40 repeat or tudor domains.[9] Using mass spectrometry-based proteomics, Vermeulen and colleagues recently performed a screen for chromatin readers at the H3K4me3.[10] They identified, with great precision, both previously known readers (e.g., the TFIID complex) as well as new interactors (e.g., PHF8, GATAD1, SGF29, TRRAP, BAP18). The novel H3K4me3 readers were all associated with promoters, coinciding with H3K4me3 mark. Two proteins were repressors, while three other proteins were part of conserved complexes associated with active transcription—SAGA/ATAC, SAGA/NuA4 and BPTF/NuRF.[11,12] The double tudor domain in SGF29 was shown to be both necessary and sufficient to mediate interaction of the SAGA complex with H3K4me3.[10] We previously showed correlation between KDM5A binding and H3K4me3 in vivo,[13] which is consistent with in vitro data that KDM5A binds tightly to H3K4me3 with a K_d of 0.75 mM.[14] While the KDM5A protein contains three PHD domains, only the C-terminal PHD domain is involved in H3K4me3 recruitment.[14] Vermeulen and colleagues found that KDM5A provides a direct link between the H3K4me3 mark and a protein complex formed by the Sin3/HDAC transcriptional corepressor complex and two poorly characterized proteins, GATAD1 and transcriptional repressor EMSY.[10] The Sin3/HDAC complex has previously been reported to interact with KDM5A in mammalian cells[15] and with the Lid complex in Drosophila.[16,17] Therefore, methylation at H3K4 is at the center of an elaborate mechanism that regulates recruitment of both activator and repressor complexes.

This suggests that a particular expression outcome may be determined by the recruitment of a site-specific reader. First, a reader (e.g., PHD fingers in mammalian BPTF and ING2 proteins) can help to stabilize the respective protein complex at the target promoter.[9] Second, histone reader domains are important for catalytic activity. Interaction between the PHD and JmjC domains of PHF8 increases the demethylation activity at H3K9me2.[18] Third, some of the PHD-containing proteins also possess enzymatic activities directed against methylated residues and thus become "reader-writers" or "reader-erasers." Studies performed in cellular and animal models showed that uncoupling of a reader from other domains can result in a potent leukemia oncogene. The *MLL1* gene is commonly rearranged and activated in multiple-lineage leukemias, including AML and ALL. KDM5A has recently been shown to be involved in a translocation in an infant with AML.[19] MLL1 and KDM5A each contain a PHD domain that interacts with the methylated lysine 4 in histone H3.[14,20] The *KDM5A* translocation results in fusion of the H3K4me3-recruiting PHD finger of KDM5A to the transcriptional activator NUP98, a common leukemia translocation partner. Recruitment of MLL1- or KDM5A-oncogenic fusions, which lack respective catalytic domain, leads to persistent gene activation.[14,21] Therefore, a hallmark of leukemia is perturbed coordination between the reading and writing or erasing events of histone methylation.

Finally, a reader communicates the bound chromatin mark with other proteins as part of an activator or repressor complex. Based on a high correlation between KDM5A localization and H3K4me3-rich genomic regions, it is highly likely that KDM5A plays a role in activated transcription, in addition to its well-anticipated role as a repressor. The KDM5 homolog in fly Lid interacts, genetically and biochemically, with Myc.[22] In contrast to its expected repressive role as a H3K4 demethylase, Lid was found to be necessary for Myc transactivator function. In *Schizosaccharomyces pombe*, the KDM5 homolog Lid2 forms different complexes.[23] In heterochromatin, where Lid2 behaves as a H3K4me3 demethylase, the complex includes the H3K9 methylase Clr4. In euchromatin, Lid2 is in a complex with Set1 which methylates K4 and Lsd1 which demethylates K9 and thus, has an activating role. This suggests that expression and availability of different complex subunits determine the alternative binding partners. While a histone mark can be targeted by multiple readers, some readers are able to bind several histone marks but only one particular mark is used for recruitment. SGF29 is able to bind both H3K4me2 and H3K4me3 in vitro.[10] However, in vivo SGF29 is associated with promoters and overlaps with H3K4me3 but not H3K4me2. This suggests additional requirements such proteins have in vivo to discriminate between di- and trimethylation. How this relates to recruitment and, finally, the activities of histone-modifying enzymes at particular genes, are exciting avenues of investigation.

Therefore, despite being associated with active transcription, H3K4me3 is attacked by both repressor and activator complexes. As chromatin consists of the largest and most complicated supercomplexes found inside the cell, the histone-modifying enzymes are brought into contact with multiple proteins. The maintenance of chromatin structure requires these proteins to restrict the distribution of histone-modifying enzymes. Data from enzymatic and structural studies suggest that multiple complex subunits and different binding domains inside each HMT/HDM enzyme influence the specificity of binding. However, in vivo mechanisms targeting the reader proteins to specific nucleosomal sites are very poorly defined. In the past two years, genome-wide studies including immunoprecipitation with massively parallel sequencing (ChIPseq) have challenged these views. This perspective focuses on recent developments in the characterization of the modes of recruitment of histone-modifying enzymes. We performed a calculation of binding site distribution and the distance of binding sites from the TSS based on the genomic position of enriched peaks from relevant publications.

trxG/PcG Regulatory System in Drosophila

Analysis of genetic data from *Drosophila melanogaster* showed that both repressed and active gene expression states tend to be inherited through successive cell cycles and throughout development. Trithorax (trxG) and Polycomb (PcG) group proteins were first identified as factors that maintain silent and active transcriptional states of homeotic (*Hox*) genes.[24] Subsequent genome-wide analysis of PcG complexes in mouse and human embryonic stem (ES) cells revealed that they also bind to genes encoding developmental regulators, thus demonstrating that PcG mechanisms discovered in Drosophila are valid in a general context. It has been known for a long time that both groups of proteins are recruited by DNA regulatory motifs several hundred base pairs long, called Polycomb and Trithorax response elements (PREs and TREs). Drosophila PREs were defined in genetic and functional tests. They induced a variegated phenotype at the mini-*white* gene and many were studied in detail. Most recently, genome-wide studies in Drosophila confirmed that PcG proteins remain closely associated with predicted PRE sequences. Strikingly, Drosophila PREs are also TREs in the sense that they are also bound by the Trithorax protein (TRX). Specifically, out of 170 computationally defined PREs, 94% bind TRX, irrespective of their gene activation status.[25] This explains the mechanism by which TRX can counteract PcG action, a phenomenon well known from genetic and functional studies. While PcG complexes may dissociate from PREs when the gene is active, TRX is bound to the PRE whether the gene is active or repressed. TRX encodes histone H3K4 methyltransferase and appears to antagonize PcG repression at the PRE and to promote active state.

The recruitment of PcG proteins involves several mechanisms which are particular to the three classes of PcG protein complexes found at PREs.[26] The first class is the PRC2 complex, which contains the SET domain-containing Enhancer of Zeste [E(Z)] subunit that trimethylates lysine 27 of histone H3. This mark is specifically recognized by the chromodomain of Polycomb (PC), a subunit of the second class of PRC1-type complexes, which also contain Polyhomeotic (PH) and several other subumits. The third PcG protein complex includes the sequence specific DNA binding protein Pleiohomeotic (PHO/YY1) and the dSfmbt protein [Scm-related gene containing four malignant brain tumor (MBT) domains], which binds to mono- and dimethylated H3K9 and H4K20. While PRC2 and PRC1 complexes do not bind DNA, PHO binds DNA in a sequence-specific manner and is able to bring PRC2 to the PRE of the homeotic *Ubx* gene in Drosophila. PRC2 binding in turn might recruit PRC1 via the methylation of K9 and K27 of histone H3, followed by recognition of these marks by the PC chromodomain.[27] However, PcG recruitment is much more complex and several recruiters, such as GAGA factor (GAF), Zeste, Pipsqueak, DSP1, Grainyhead and members of the SP1/KLF family, may be required in vivo. Two PRE motifs, defined as PHO and GAF motifs, seem to be necessary but still not sufficient for PcG recruitment at PREs. Surprisingly, the addition of a different motif, 5'-GAAAA-3' (G/A), to a heterologous DNA sequence was sufficient to recruit the PcG proteins PHO, E(Z) and PH, although weaker binding was observed.[28] The G/A motif was shown to be bound by the Drosophila homolog of HMGB2, Dorsal switch protein 1 (DSP1). Mutations in DSP1 that abolished binding to this PRE resulted in the loss of PcG protein binding and loss of silencing. Still, while these proteins often bind near known PREs, the number of their binding sites differs greatly and none of them was found to be present at all regions bound by PcG. This indicates that multiple DNA sequences contribute to protein binding. Additional heterogeneous sequences may indirectly influence the binding through an effect on chromatin architecture.

While PREs have been defined only in Drosophila so far, HMT and HDM binding sites discovered in mammals might fit this criterion as well. Considering the role of MLL1 in the regulation of *HOX* genes and the conservation in PcG complex function in flies and mammals, the existence of PRE/TREs can be proven by transgenic assays in mammalian cells. It is possible that mammalian and fly PRE/TREs will have some DNA motifs in common. It will be important to determine whether PcG and trxG bind sites simultaneously or alternate over time. The contributing DNA binding factors will be different, since some of these proteins, including GAF, Zeste and Pipsqueak, are not evolutionarily conserved. Another difference will be in the role of the pattern of histone marks. Mouse and human PRC2 components bind throughout H3K27me3 regions,[29,30] whereas Drosophila PRC2 members bind to restricted regions in a mechanism involving a looping model.[31,32] The degree to which HMT/HDM function in mammals differs from the trxG/PcG function in Drosophila is a largely open question. There is sufficient evidence that argues against yeast Set1 and mammalian MLL1 being functional orthologs of TRX. In a recent study by Schwartz and colleagues,[25] TRX was found at PREs and only at a small subset of active genes marked by H3K4me3 (defined as regions with RNA polymerase II (RNA Pol II) at the Transcription Start Site (TSS) and H3K4me3 at position +500 bp). Consistent with the absence of TRX recruitment at H3K4me3 regions, there was no decrease in the overall level of H3K4me3 after *trx* RNA inhibition. In contrast, PcG target regions were enriched in H3K27me3. The *E(z)* knockdown resulted in prominent reduction in global H3K27me3 levels, as expected. Moreover, the *KDM5A* homolog *lid* by genetic criteria belongs to *trxG* but not to *PcG* genes. *lid* was identified in a screen for *trxG* mutations on the basis of intergenic noncomplementation with the *ash1* allele.[33] *lid* also showed enhancement of the mutant phenotype of other *trxG* genes, *brahma* and *trx*, and suppressed the phenotype of *PcG* mutations. The search for evidence of a role for KDM5A in trxG/PcG regulation has yielded data showing that KDM5A is recruited to the promoters of *HOX* genes and that its knockdown by RNAi affects *HOX* gene expression.[14,34] Additionally, altering KDM5A expression causes developmental abnormalities in Drosophila and *Caenorhabditis elegans*.[33-35]

ChIP experiments have shown that binding of PcG proteins to their target genes in mammals appears quite dynamic.[36,37] Therefore, the "stringency"

of epigenetic memory of transcriptional programs in mammals is different compared to Drosophila. While the prominent role of PcG proteins in stem cell identity in mammals suggests they make a significant contribution to epigenetic memory in this system, in differentiating cells they may not play as big a part. This suggests that in mammals histone-modifying complexes are involved in reversible gene expression while other epigenetic modifications such as DNA methylation are responsible for epigenetic memory. The larger protein families in mammals create opportunities for precise regulation and new roles in transcription. It will be interesting to determine whether high levels of expression of particular HMTs or HDMs in some tissues correlate with their essential role in these tissues. It is tempting to speculate that a HDM-mediated removal of the methylation mark placed by a HMT could be an important part of a specific mechanism by which HMT activation is fine-tuned or counteracted. Heterogeneity within both mammalian HMTs/HDMs and Drosophila trxG/PcG proteins underlies multiple recruitment mechanisms that are adjusted to differences in chromatin organization in these organisms.

KDM5A/RBP2

One of the earliest genome-wide target analyses of HDMs was done on the Lid human ortholog KDM5A/RBP2.[13] RBP2 is a member of a protein family containing three other proteins, KDM5B, KDM5C and KDM5D.[38] RBP2 is an especially interesting HDM as it is a critical target of the pRB tumor suppressor protein during differentiation.[39] To identify differentiation-dependent and differentiation-independent targets, ChIP-on-chip analysis was done in the human lymphoma U937 cell line at various stages of differentiation.[13] In the differentiated condition, RBP2 targets were found to be enriched for genes representing distinct functional categories from the undifferentiated condition. Another study performed in mouse ES cells showed over-representation of similar gene ontology (GO) functional categories.[40] In general RBP2 targets are localized in the mitochondrion, nucleus, ribonucleoprotein complex and function in DNA repair, transcription, cell proliferation, cell differentiation, RNA processing and cellular metabolism. In both studies, RBP2 was found to bind at the proximal promoter region and highly correlated with H3K4 trimethylated nucleosomes.[13] During differentiation RBP2 is recruited to new genes which function in the mitochondrion as well as cell cycle genes. However, in contrast to "constitutively" bound genes in cycling cells which are highly expressed, the binding at these genes correlated with transcriptional repression and subsequent dissociation of the RBP2 protein from target promoters. This suggests the existence of different recruitment mechanisms at different subsets of target promoters.

In order to learn more about common features and differences in the recruitment of various histone-modifying enzymes, we determined the distribution of their binding sites and the distance of these binding sites from known genes. We used available data on the genomic positions of enriched peaks for RBP2 and the five best-studied proteins involved in histone methylation. We found that, strikingly, most of the RBP2 peaks overlap the TSS, with about 95% of the peaks being within a 1 Kb interval around the transcription start site (TSS) (**Table 1**). Because H3K4me3 marks are found at the TSS regions of active genes and RBP2 target genes highly correlate with H3K4me3, this histone mark may serve as a recruiting module in vivo. RBP2 contains an ARID domain that binds to a DNA CCGCCC motif,[41] which might be one of the contributing factors to site-specific recruitment. In addition, transcription factor binding site studies showed that RBP2 recognizes regions bound by TFs like E2F and pRB family members. RBP2 is replaced by pRB at some cell-type specific genes, while at cell cycle genes RBP2 may potentiate repression by RB/E2F complexes.[13,15] Since RBP2 interacts with a great variety of proteins, their binding to DNA or chromatin may represent a general phenomenon for RBP2 recruitment to multiple genes. One of the most recent examples is a repressor complex with RBP-J, that interacts with PHD2 and PHD3 domains of RBP2.[42] Protein-protein interaction studies have implicated pRB, TATA-binding protein (TBP), Rhombotin-2[43,44] and the recently described GATAD1, MRG15, EMSY, PHF12, SIN3B, RBAP48 proteins.[10] Motif finding algorithms in RBP2 binding promoters have revealed binding sites for the first three proteins as well as additional sites for PAX6,[13] which is not known to be a RBP2-interacting protein. Some of these interactions may influence recruitment through shaping the nucleosome density pattern, such as Sin3 binding to downstream promoter regions which is stabilized by its interaction with E2F4 and hypoacetylated histones.[15]

RBP2 is one of the few studied proteins where the reader domain and the catalytic domain are contained within the same polypeptide. Specifically, RBP2 has the 2OG oxygenase (JmjC) catalytic domain and a 60 amino acids—long PHD finger that binds to H3K4me3. The PHD finger is repeated two times in the short isoform and three times in the long isoform of RBP2,[38] and the last PHD finger (PHD3) is a reader of K4 methylation status.[14] Wang and colleagues solved the X-ray structure and ensembled NMR structures of RBP2 PHD3 in complex with the H3K4me3 peptide. This domain of RBP2 is a part of an oncogenic fusion protein and H3K4me3 binding by the PHD3 was crucial for leukaemogenesis. Expression of this fusion prevented the removal of H3K4me3 at many loci encoding lineage-specific transcription factors (e.g., *HOXA9*, *HOXA10*, *GATA3*, *MEIS1*, *EYA1* and *PBX1*) in the course of differentiation. This promoted their active transcription in less differentiated cell types, while mutations in PHD3 which abrogate H3K4me3 binding abolished leukaemic transformation. In addition, PHD3 may be essential for tethering of other transcription factors to chromatin, which was proposed for Lid and Myc interaction.[45] Whether RBP2, similar to several other known HDMs, can be recruited to methylated lysines of other proteins besides histones is an open question. This would add complexity to the non-histone protein function of RBP2 and in a disease condition, like leukemia, may contribute to pathogenesis.

KDM5B/PLU1 Occupies KDM5A Targets

Multiple studies have indicated that overexpression of HDMs result in a gross loss

Table 1. Features of HDM and HMT binding sites

Genome-wide ChIPseq and ChIP-on-chip data analysis						
HDM/ HMT	**Cell Line/Species**	**Distribution of binding sites***	**Distance of binding sites from TSS***	**Motif/binding site**	**Correlation with histone modifications**	**Other TF/ complex interactions**
KDM1A/ LSD1	primary foreskin fibroblast/human[70]	~1/2 in promoter region ~1/3 in introns and exons	~1/2 within ± 1 Kb ~3/4 within ± 2 Kb	GC-rich regions [CT]CC[AC]G[CG]	H3K4me2	EZH2, SUZ12, HOTAIR
KDM5A/ JARID1A/ RBP2	diffuse histiocytic lymphoma cell line U937 and osteo-sarcoma cell line SAOS-2/human[13] ES cells/mouse[40]	most of the peaks overlap TSS, few are inside the gene body	~95% within ± 1 Kb	[AGC][CGA][CA] GGAA[GAC][TC]G	H3K4me3	pRB, TBP, Rhombotin-2, GATAD1, MRG15, EMSY, PHF12, SIN3B, RBAP48, RBP-J
KDM6A/ UTX	primary fibroblast/ human[74]	promoter region, >50% upstream of TSS ~30% inside gene body	~70% within ± 4 Kb	N/A	H3K4me2, relatively depleted of H3K27me3	MLL2-4
KMT2A/ MLL1/ HRX	precursor B-cell ALL patient-derived cell line REH/human[21]	~1/2 overlap TSS, ~1/4 in upstream promoter ~1/5 inside the gene body	~55% within ± 1 Kb; ~65% within ± 3 Kb	N/A	H3K4me3	elongation complex (e.g., pTEFb, ENL, DOT1, PAF)
KMT6/ EZH2	ES cells/mouse and ES cells/human[37]	~1/3 in promoter region ~1/4 overlap with TSS ~1/5 inside gene body ~1/5 downstream of TSS	~1/2 (peaks are described as HMM intervals) within ± 3 Kb	CpG islands and GC-rich regions	H3K27me3 H3K4me3	SUZ12, MTF2, JARID2, EED
JARID2	ES cells/mouse[40]	~2/3 overlap with TSS	~70% within +/-3 Kb	CpG islands CCG and GA-rich regions [CTG][CTA]GC[CTA] [GA]C[CTAG][GAT] C[CTA][GAT]C[CTG] [GCT] [GCA]A[GA][GCA] [ATC][GA][CGA] [ATC][GAT][GCA] [AGC][GAC][GCA] [ATC]G	H3K27me3; bivalent H3K4me2/ H3K27me3	EZH2 and SUZ12 (>90% of targets), MTF2

This Table combines HMT and HDM data available from genome-wide studies. Data for the protein highly homologous to KDM5 enzymes, JARID2, was also included. When multiple studies were performed, we gave our preferences to ChIPseq data from human cells. TF, Transcription Factors; TSS, Transcription Start Site. Motifs are presented in regular expression. When more than one nucleotide is probable in the same position, they are mentioned in square brackets starting from higher bit nucleotide to lower bit nucleotide. N/A, information not available. *Distribution of binding sites and distance from TSS was calculated as described in Materials and methods.

of global histone methylation.[38] Often, however, gene knockdown and knockout result in quite minor changes. Analysis of *Rbp2* knockout mice in a mixed genetic background showed no obvious abnormalities.[46] Since the RBP2 protein family has three other members, a plausible explanation for this can be functional compensation from other KDM5 proteins. KDM5C and KDM5D are closely homologous sex chromosome-specific genes and thus represent unlikely candidates. KDM5B, in contrast, is co-expressed with RBP2 in multiple cell types and has a similar domain organization. In particular, unlike KDM5C and KDM5D, it contains a PHD3 domain, which in RBP2 has shown to be the H3K4me3-recognition module. We investigated the possibility of functional compensation for the lack of RBP2/KDM5A by the KDM5B protein in both gene knockdown and knockout studies. ChIP-on-chip analysis showed that RBP2 can be detected at promoter regions of multiple genes.[13] We designed primers to RBP2 target genes to analyze the extent to which KDM5A binding can be compensated by the binding of KDM5B. The RBP2 target genes were randomly selected from our genome-wide RBP2 occupancy data in SAOS-2 cells.[13] We used chromatin immunoprecipitation followed by real-time PCR (ChIP-qPCR) with selected primers to compare enrichment of both proteins. Consistent with ChIP-on-chip data, all regions in the analysis demonstrated an increased RBP2/KDM5A level compared with the control unbound region (**Fig. 1A**). In contrast, no KDM5B binding was detected at these regions. However, the KDM5B was recruited to multiple RBP2 target regions in *KDM5A* siRNA-treated cells compared with control siRNA treated cells (**Fig. 1A**). We next asked if a complete absence of RBP2 would potentiate KDM5B recruitment as well. We used wild-type and *Rbp2* knockout mouse embryonic fibroblasts (MEFs)[46] to study KDM5B binding at the regions normally bound by RBP2/KDM5A. We were able to detect KDM5B enrichment in *Rbp2*$^{-/-}$ MEFs at the regions that are occupied by KDM5A in wild-type MEFs (**Fig. 1B**). These suggest that

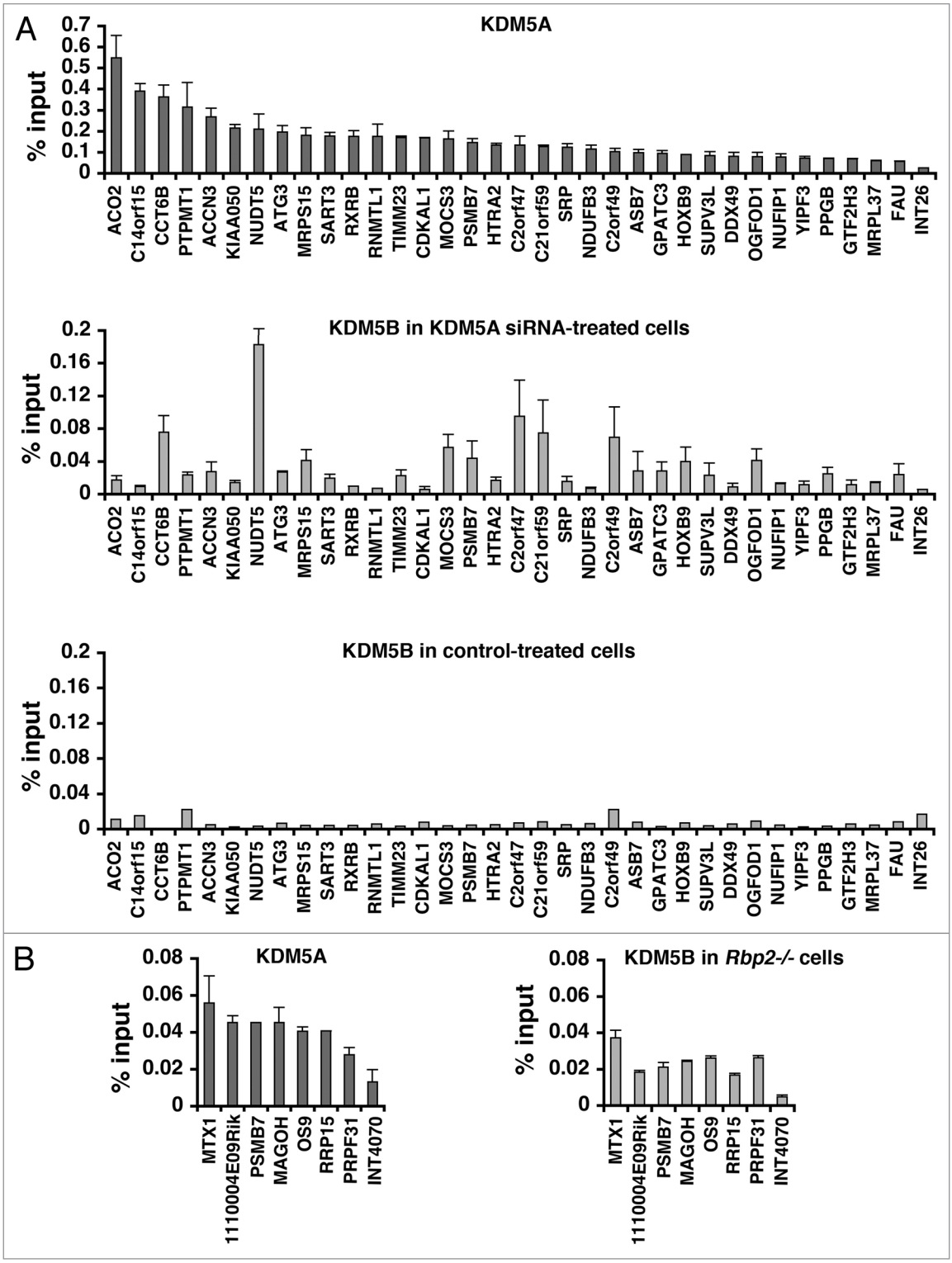

Figure 1. KDM5A deficiency results in KDM5B recruitment to KDM5A targets. (A) KDM5A knockdown by RNAi results in KDM5B recruitment to KDM5A targets. The ChIP-qPCR experiments of KDM5A and KDM5B binding were performed at the KDM5A target genes indicated on the bottom of the graphs. These KDM5A target genes were randomly selected from the genes defined in our previous ChIP-on-chip experiments with KDM5A antibodies in SAOS-2 cells as RBP2/KDM5A targets (p value < 0.002).[13] The ChIP experiments were performed in mock SAOS-2 cells, SAOS-2 cells treated with KDM5A siRNA or with control siRNA. The obtained values were reproducible in two independent experiments. (B) KDM5B is recruited to KDM5A targets in *Rbp2*$^{-/-}$ MEFs. Genes with the highest enrichment of KDM5A in ChIP-qPCR (% input) relative to the unbound human and mouse control intergenic regions, INT26 and INT4070, respectively, are shown at the right of the graph. Error bars represent standard errors calculated from two ChIP experiments.

these regions can be specifically distinguished by both KDM5A and KDM5B. These findings also suggest that the effect of *RBP2* siRNA may reflect KDM5B function at some RBP2 target promoters. Since our previous microarray studies showed that KDM5A targets are not generally repressed,[13] they point out that KDM5B function at these targets does not involve repressing HDM activity. These data indicate that the propensity of histone demethylases to switch targets may depend on the relative level of functional homologs. Studies of a double knockout of RBP2 and KDM5B will probably be helpful in distinguishing the role of KDM5B.

KMT2A/MLL1

One of the mammalian TRX's homologs is MLL1. It exhibits the opposite regulatory effect of KDM5 (by methylating rather than demethylating H3K4) and the mammalian catalytic subunit of PRC2, Enhancer of zeste homolog 2 (EZH2) (by methylating K4 rather than K27 of histone H3). The C-terminal portion of MLL1 contains the SET domain which "writes" methyl marks on H3K4.[9] MLL1 is a large nuclear protein processed by the taspase 1 protease and cleaved into a 300 kDa N-terminal fragment (MLLn) and a 180 kDa C-terminal fragment (MLLc). MLL1 and its family members have a common core complex containing ASH2L, RBBP5 and WDR5 proteins.[9] Despite sharing a catalytic domain and core complex subunits, MLL proteins regulate different genes.[47,48] This results in different functional outcomes, such as the MLL2 protein fusion being unable to transform bone marrow cells in MLL1 domain swapping experiments.[49]

H3K4 methyltransferase activity was first described for the *Saccharomyces cerevisiae* Set1 protein.[50] Set1 was shown to co-localize with RNA Pol II in the region immediately downstream the TSS of highly expressed genes.[50,51] The Set1 localization creates epigenetic memory because the H3K4 trimethylation in the 5' coding region is retained long after transcription has ceased. Similar to Set1, MLL1 also localizes to the 5' region of actively transcribed genes marked with H3K4me3 (**Table 1**). Our computational analysis showed that MLL1 mostly localized close to the TSS, spanning on average a 1–3 Kb region of the 5' end of the transcribed portion of the gene.

The hallmark function of the MLL family of HMTs is maintaining expression of developmental genes such as *HOX* genes.[52] Proper regulation of *HOX* gene expression is critical to normal embryogenesis and normal hematopoietic differentiation. The MLL family has a prominent role in regulating *HOX* gene expression in hematopoietic cells to establish cellular identity. This is particularly significant because misregulation of *HOX* genes is associated with the onset of leukemia phenotypes in patients with chromosomal translocations in the *MLL1* locus.

Studies of the MLL1 fusion proteins in leukemia provided insights into the biological role of different recruitment mechanisms. MLL1 occupies an extensive chromatin domain at the *HOX* genes, in contrast to the majority of genes.[53] Milne and colleagues mapped MLL interactions required for recruitment to the critical leukemia target *HOXA9*.[20] *HOXA9* gene is normally expressed in haematopoietic stem/progenitor cells and gets repressed as differentiation progresses, while it is continuously expressed in MLL1-rearranged human leukemias.[54,55] *HOXA9* can have H3K4me3 and H3K27me3 modifications spread across the entire locus and thus, represents a classical bivalent locus. When the MLL1 complex binds to the *HOXA9* gene, it causes gene activation through elimination of the repressive marks H3K9me3 and H3K27me3. A minimal MLL1 recruitment domain at *HOXA9* locus requires the PHD3 finger (out of the four PHD fingers of MLL1) and the CXXC region (see below).[20] The PHD3 finger binds directly to H3K4me2/me3. MLL1 fusion proteins, like MLL-AF9, lack the PHD fingers. However, the MLL1 fusion proteins retain the CXXC domain, which represents a "weak" recruiting module to *HOXA9* gene. MLL's CXXC region mediates direct interaction with the PAF1 complex. PAF1 was found to be required for stable binding of both WT MLL1 and MLL1 oncogenic fusions and for H3K4 and H3K79 methylation at *HOXA9*.[20,21] Specifically, a point mutation in the CXXC domain that disrupted the PAF1 interaction but had no effect on DNA binding, almost completely disrupted MLL1 recruitment to the *HOXA9* promoter.

Mammalian H3K4 HMTs were also well-studied for the role played by DNA modifications in forming their binding sites. MLL1 has a prominent role in DNA methylation because it can prevent and stabilize it, directly or indirectly.[56-58] If direct interactions are involved, then H3K4 HMTs would be expected to bind unmethylated CpGs. This interaction has now been confirmed by a solution structure of an MLL1-CXXC domain in a complex with unmethylated DNA and by specific mutations disrupting interaction with unmethylated DNA which showed the predicted effects.[59] SETD1A, while lacking its own CXXC domain, interacts with a protein that contains this domain, the CXXC finger protein 1 (CFP1). Consequently, cells deficient for *CFP1*, which exhibit a decreased level of global DNA methylation, also exhibit an elevated global level of H3K4me3.[60] Furthermore, point mutations in *CFP1,* specifically eliminating DNA binding or SETD1A interaction, also severely compromised the ability of the *SETD1A* alleles to complement the phenotype of the *CFP1* deletion. The same mutants had also compromised SETD1A distribution which is normally limited to euchromatin.[61]

It is recognized that recruitment of epigenetic factors is a part of the cascade of events underlying cell-type specific gene activation. p38 mitogen-activated protein-kinase (MAPK) signaling was shown to regulate recruitment of MLL complexes to muscle-specific genes.[62] In growing myoblasts, muscle-specific genes are epigenetically silenced by H3K27me3. The PcG protein EZH2 is recruited to their promoter regions by YY1, a homolog of Drosophila PHO.[63] Soon after induction of differentiation, MEF2 is recruited to the *MYOG* promoter. MEF2D gets phosphorylated by the p38 MAPK, which promotes its interaction with ASH2L, a MLL core complex component.[62] siRNA experiments showed that ASH2L was recruited to muscle-specific genes through a MEF2-dependent mechanism. Specifically, when promoter binding of MEF2 was reduced by 80%, ASH2L binding was reduced in

half. Treatment with a p38 kinase inhibitor showed that the kinase activity was required in vivo for maximal recruitment of ASH2L complexes and H3K4 trimethylation at target promoters.

Therefore, MLL recruitment can be accomplished through interactions with H3K4me2/me3, PAF1, cell-type specific transcription factors and DNA, which together are able to stabilize MLL binding. Surprisingly, genome-wide location analysis of wild-type (WT) MLL1 and MLL-AF4 in a precursor B-cell line from an acute lymphoblastic leukemia patient showed that MLL-AF4 binding may mirror WT MLL1 binding at the *HOXA9* locus and cause an increase in H3K4me3 and H3K79me2 levels.[21] However, these data are consistent with the fact that MLL1 translocations usually involve only one allele of MLL1-leaving another allele of MLL1 intact[64] and that MLL-AF9-induced leukemogenesis requires coexpression of the wild-type *MLL1* allele.[65] So, the MLL1 fusion protein can be recruited through CXXC domain interactions with PAF1 and DNA, however, requires the presence of wild-type MLL1. It is plausible that the WT MLL1 prebound at the *HOXA9* locus creates an "open" chromatin state, providing a mechanistic explanation for its requirement in leukemia.[65] It is also possible that several proteins that are shown to be important for MLL1 recruitment to *HOXA9*, such as menin and LEDGF/p75,[65,66] interact with WT MLL1 and recruit MLL1 fusion protein.

KDM1A/LSD1

LSD1 is the first discovered histone lysine demethylase and belongs to the flavin adenine dinucleotide-dependent enzyme family.[67] The LSD1 homolog in Drosophila is required for proper repression of *Hox* genes.[68] Repression by LSD1 is partially mediated by its interaction with repressors, for example with the CoREST complex which bridges LSD1 with the repressor element 1 silencing transcription factor REST.[69] Genome-wide KDM1A/LSD1 studies performed in human primary foreskin fibroblasts showed that nearly one third of LSD1 bound promoters were occupied by SUZ12, a component of the PRC2 complex.[70] The regions were co-occupied by LSD1 and SUZ12 at a distance of less than 500 bp on average and displayed GC-rich motifs. Such significant overlap is consistent with the REST motif being one of the prominent DNA sequences within PRC2 binding sites.[37] LSD1 displays more than half of its binding sites within 1 Kb from the TSS (**Table 1**). Both PRC2 and LSD1 can bind multiple proteins, which may provide DNA binding specificity to the PRC2/LSD1 supercomplex. This interaction was mainly mediated by a long intergenic noncoding RNA (lincRNA) HOTAIR which served as a scaffold to bring the two complexes, PRC2 at the 5' end and the LSD1/REST repressor complex at the 3' end, together.[70] Also co-immunoprecipitation experiments showed interaction of LSD1 with another PRC2 complex member, EZH2. At PRC2 targets, LSD1 recruitment was HOTAIR dependent and commonly occurred in CpG islands. Consequently, HOTAIR knockdown led to a concordant loss of SUZ12 and LSD1 occupancy at about 40% of target genes. The knockdown resulted in the loss of a GC-rich motif, which is important for PRC2 binding in regions occupied by LSD1. It is conceivable that the targeting of both PRC2 and LSD1 proteins to chromatin couples both histone H3K27 methylation and histone H3K4 demethylation.

As many as 6,570 promoters (34% of the array) were identified in a previous ChIP-DSL LSD1 study.[71] This study was performed in the ER-positive breast cancer cell line MCF7. However, in contrast to its role in repressor complexes, LSD1 was found to bind most of the ERα targets in MCF7 cells. As expected, after treatment with 17β-estradiol, LSD1/ERα-dependent gene targets experienced increased ERα binding and increased H3K9 acetylation, but also showed a consistent decrease in both H3K4me2 and H3K9me2. Thus, besides its role in repression, LSD1 is involved in transcriptional activation. Consistent with these data, full activation of androgen receptor (AR) targets required both LSD1 and the H3K9 histone demethylase JMJD1A.

KDM6A/UTX

The H3K27-specific histone demethylases are represented by three jmjC domain proteins, UTX, UTY and JMJD3. Analogous to the association of LSD1 with PRC2, UTX was found to interact with the H3K4 HMTs MLL2-4.[72,73] At some targets, MLL2 was shown to even be required for UTX recruitment.[72] Therefore, through UTX, H3K4 methylation might be coordinated with H3K27 demethylation. H3K27me3 is found at permanently silenced genes, such as those involved in functions irrelevant to a particular cell type. H3K27me2 has a similar distribution to H3K27me3, though less biased toward silent genes and virtually ubiquitous in euchromatin. H3K27me2 is highly abundant, comprising more than 50% of total histone H3. H3K27me2/me3 are deposited by EZH2. Although the effect of H3K27me2 on transcription and on H3K4me3 has never been studied, should it turn out to be negative we would expect that the removal of the H3K27 methyl mark would be required at virtually all promoters regulated by MLL complexes. A genome-wide study in primary human fibroblasts reported that the majority (62%) of 1945 identified UTX targets are enriched for the univalent H3K4me2 mark, a few percent showed the H3K27me3 mark, and some showed bivalent H3K4me2/H3K27me3 marks.[74] In most cases UTX occupied upstream regions from the TSS (**Table 1**). Gene set enrichment analysis performed on subsets of UTX genes showed that targets with H3K4me2 were enriched for multiple GO terms such as biogenesis, chromosome organization and RNA splicing; targets with H3K27me3 marks were enriched for GO term developmental process; targets with both histone marks were enriched for a group of cell adhesion proteins; lastly, targets with neither of these marks were enriched for olfactory receptors. Consistent with inactivation of UTX by somatic mutations in cancer,[75] UTX removes H3K27me3 at several genes involved in the RB tumor suppressor pathway.[74] Importantly, this was shown to be essential for regulation of cell fate in an RB-dependent manner.

KMT6/EZH2

In mammals, as in Drosophila, PcG complexes play an important role in the regulation of developmental genes. They are

represented by the conserved Polycomb Repressive Complexes 1 and 2, PRC1 and PRC2, which share a majority of target genes. The complex containing the HDM subunit EZH2, PRC2 (also contains PcG proteins SUZ12 and EED and the nucleosome binding protein RBBP4/RbpAp48), implements transcriptional repression by catalyzing the di- and trimethylation of H3K27. Inhibition of EZH2 by RNAi results in a global decrease in the levels of both di- and trimethylation at H3K27 suggesting that EZH2 is the major H3K27 methyltransferase.[76,77] Genome-wide analysis of EZH2 was performed in several studies in mouse ES cells.[37,40] Similar to the enzymes involved in H3K4 methylation, EZH2 occupies many sites within 3 Kb from the TSS (**Table 1**). Genomic sites of PcG group proteins were found to be enriched not only for the repressive H3K27me3 mark but also for the activating H3K4me3 mark.[37] In total, roughly 3,000 mouse and 2,500 human genes in ES cells carry the H3K4me3/H3K27me3 "bivalent" marks. As ES cells differentiate, many bivalent promoters loose the repressive H3K27me3 mark while retaining the "univalent" activating H3K4me3 mark.[6,7] On the other hand, non-induced genes retain the H3K27me3 mark and loose the active H3K4me3 mark. This seems to represent a general phenomenon as bivalent marks are not limited to developmental genes or to stem cells.[37,78]

In fact, most of the bivalent marks overlap with PRC2 sites and there is a significant overlap with both PRC2 components, EZH2 and SUZ12. Another feature of the bivalent marks and PcG targets is enrichment for GC-rich DNA. Strikingly, 97% of Ezh2 targets are found in CpG islands or in GC-rich DNA regions. The H3K27me3 mark was shown to be specifically recognized by the chromodomain of the PC protein in the PRC1 complex.[79] The recruitment of the PRC1 complex is, to a large extent, dependent on the recruitment of the PRC2 complex, however, it is not very well defined what determines the specificity of the primary recruited PRC2 complex.[80] The observation that the PRC2-targeted CpG islands are enriched for binding motifs of transcriptional repressors, NRSF/REST and CUX1, suggests that transcription factors localized to GC-rich regions may contribute to PRC2 targeting.

Protein interaction and binding site studies showed that besides PRC2 core components, EZH2 interacts with JARID2, forming a stable supercomplex on chromatin.[40,81-85] The ARID domain of JARID2 can directly bind to DNA, holding the promise for JARID2 to act as a recruiter. Indeed, recruitment of PRC2 is ARID domain dependent.[83] JARID2 is sufficient to recruit PcG proteins to a heterologous promoter. Target sites of SUZ12, EZH2, EED and also MTF2, another PRC2 interacting protein, heavily overlap with JARID2 targets in mouse ES cells.[40,81,83,85] Knockdown of JARID2 leads to a major loss of EZH2 and SUZ12 binding sites, which is consistent with the fact that the majority of PRC2 in ES cells is bound to JARID2. However, JARID2 effects on PRC2 HMT activity and H3K27me3 levels may depend on target promoters.[40,83] JARID2 plays a crucial role in the differentiation of mouse ES cells, which is consistent with an essential role for PcG proteins in early development. Importantly, both JARID2 and PcG proteins showed highly significant enrichment in target gene categories associated with development, morphogenesis and transcription. This suggests that JARID2 and PcG proteins share some biological functions.

JARID2

The JARID2 protein is not considered to be a histone demethylase, due to the lack of two conserved histidine residues in the HXD/EXnH metal-binding motif of the JmjC domain. In fact, JARID2 regulates histone H3K27 HMT activity, but probably by a non-enzymatic mechanism.[40,81] Despite a lack of enzymatic activity, JARID2 is crucial for the differentiation of ES cells. Specifically, SUZ12 interacts with the amino acid region 726–913 in JARID2.[40] Interestingly, the amino acid motif "GSGFP" is conserved in JARID2 and the KDM5A protein and responsible for recognition of SUZ12 by both JARID2 and KDM5A. Yet, in sharp contrast to JARID2, there is no significant overlap between KDM5A and PRC2 loci.[40] Consistent with these data, there is a significant overlap of KDM5A with H3K4me3 regions but not with H3K27me3 regions,[13] providing a strong argument against KDM5A forming a chromatin bound complex with PRC2. In contrast, JARID2 binding sites highly correlate with H3K27me3 binding sites.[40,83,85] They are located close to the TSS, on average ±3 Kb (**Table 1**). Similar to PcG targets, a CCG motif and a second GA-rich motif were common for JARID2 binding. Moreover, the relationship between PRC2 and JARID2 is mutual and stability of JARID2 binding is dependent on the integrity of the core PRC2 complex.[81] The PRC2/JARID2 supercomplex formation is probably facilitated by other transcription factors and non-coding RNAs.

Combinatorial Binding Results in Specific Recruitment

One of the crucial questions in epigenetic regulation is whether DNA and modification of histones are a driving force for recruitment of chromatin-associated proteins capable of histone modifications. Is a "reader" domain necessary or sufficient to target the "writer" or "eraser" to the right place on chromatin? Here we showed that KDM5B bound to the sites of its homolog, KDM5A, in cells deficient in KDM5A. Therefore, in normal condition KDM5B is restricted from binding these targets by KDM5A or KDM5A interacting proteins. This suggests about tissue-specific reciprocal binding of related histone-modifying enzymes at some gene targets.

Gene knockdown studies in Drosophila showed that TRX/ASH1 and PcG do not exist in a competitive equilibrium for their binding sites.[25] Also, studies of PRE motifs in Drosophila showed that there is no definitive signature of trxG and PcG binding sites. The recruitment of trxG and PcG complexes is believed to depend on DNA-binding proteins that directly interact with or facilitate their binding. In fact, domain swapping experiments demonstrated that the preference of a given reader domain for a particular methylated residue in vitro is not reflected in the sites enriched for this mark in vivo.[86,87] Thus, the most likely scenario is that histone-modifying proteins can be recruited in multiple ways, by different combinations of proteins.

The histone-modifying proteins can form supercomplexes themselves, such as the complex comprised of HOTAIR, PRC2 and LSD1, which enables their coordinated binding to target regions. Once bound to DNA, they can in turn initiate a complicated cascade of recruiting events. PHO might recruit the PRC2 complex, as well as PRC1 components, by direct protein-protein interactions as well as induction of appropriate histone marks.

Interestingly, some results suggest there is cooperation in the recognition of histone H3K4 methylation and histone H3 acetylation in chromatin readers. TFIID and BPTF bound more strongly to the H3K4me3 mark when it was flanked by acetylation on H3K9 and H3K14. There are several mechanisms that can lead to this outcome. A PHD finger domain can be combined with a bromodomain in one protein as in BPTF. Both domains can be brought together in a protein complex such as in the TFIID, which contains a PHD finger of the TAF3 subunit and TAF1 bromodomains. Moreover, a domain different from PHD can be used, such as a Tudor domain in SGF29 in the SAGA complex, which also contains a GCN5 bromodomain. As H3K4me3 and H3K27 acetylation increase at the promoter, with the highest peaks around 100 base pairs downstream of the TSS,[3] it can create the preference for SGF29 recruitment to nucleosomes associated with H3K4me3 rather than H3K4me2.

We have considered "DNA code," histone code and interacting proteins as factors contributing to gene-specific recruitment, while leaving small RNAs and proteins of the RNAi machinery outside the scope of this perspective. Also, the transcriptional activity of the target locus per se, as a reflection of activity of such factors, plays a role. This is beautifully exemplified in studies of heat shock genes that can become rapidly activated by heat shock factors. TRX was purified from Drosophila embryos as a component of the TAC1 complex. Promptly after a heat shock, TAC1 is recruited to several heat shock gene loci, where its components are required for high levels of expression.[88,89] TAC1 promotes transcription of heat shock genes in a mechanism that includes association with transcription-competent poised or stalled RNA Pol II and methylation and acetylation of histone H3. Such modifications of histone H3 facilitate transition of the stalled RNA Pol II to the elongating form. Consequently, recruitment of the TRX is affected by mutations in the FACT elongation complex.[90] In budding yeast, the H3K4 HMT Set1 is a component of the COMPASS complex, which is recruited by the PAF elongation complex at actively transcribed genes.[51,91] The requirement for its interaction with the elongation complex at specific genes is unclear. *Hox* genes do not have poised or stalled RNA Pol II, suggesting that a distinct mechanism is employed from that of heat shock genes.

Important insights have come from studies on recruitment of MLL1 and its oncogenic fusions. The wild-type and oncogenic MLL1 fusion proteins have both overlapping and distinct recruitment mechanisms. MLL1 binding is affected by the presence of H3K4me2/3, while binding of the MLL-AF9 fusion protein in leukemia cells depends solely on DNA binding and PAF interaction. Binding of MLL1 to either methylated H3K4 or PAF alone may not be sufficient for recruitment, but both nucleosomal and PAF complex interaction together could result in stable and specific recruitment of MLL1 to *HOXA9*. Overall, loss of MLL function through both haploinsufficiency and gain-of-function effect may contribute to leukemogenesis.

We support the hypothesis by Ruthenburg and colleagues[9] that histone-modifying enzymes are recruited to their genomic targets through multiple interactions, which are transient and weak when work alone but, all together result in specific and stable binding of the factor to chromatin. Does the combination of "reader" with "writer" or "eraser" domains in the same protein result in the coupling of histone modification with recruitment? In cases where there are modules within the same polypeptide for recognizing and writing the same mark, such as H3K9me1/me2 methyltransferase KMT1C/G9a, the ankyrin repeats and the SET domain create cross-talk which leaves a particular methyl mark.[92] In the cases of two opposing marks, this would result in cross-talk to remove an "OFF" methyl mark based on an existing "ON" methyl mark. For example, PHF8 and JHDM1D contain modules for recognizing (via the PHD) and removing (via the JmjC domain) histone marks.[93] While the coupling of enzymatic reaction to recruitment may be true for some enzymes, it is not evident for others. While just a few percent of targets of the H3K27 HDM UTX show H3K27me3 mark, targets of the H3K4me3 HDM KDM5A heavily overlap with its substrate. One possibility is that while UTX recruitment results in simultaneous demethylation, demethylation activity of the recruited KDM5A is poised. Importantly, on the genes where we were able to see differential KDM5A binding, the binding event indeed correlated with a loss of methylation. However, these results do not exclude the possibility that at constitutively occupied genes KDM5A may instead or in addition have a general role in activated transcription.

Studying novel aspects of histone modifying enzymes should lead to de novo analysis of all genes whose expression depends on their recruitment. These include analysis of DNA motifs and their combinations, posttranslational modifications of histone-modifying enzymes, structural and functional analyses of different HMT and HDM isoforms and their oncogenic fusions. It will also aid in development of targeted therapies in which the specifically inactivated recruitment domain would be more disruptive to an oncogenic protein than the normally functioning protein.

Materials and Methods

Chromatin immunoprecipitation, ChIP-qPCR. SOAS-2 osteosarcoma cells and MEFs were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). The siRNA experiment with RBP2 siRNA 4 (for KDM5A knockdown) and ChIP assays were performed as described previously.[13] Two rabbit affinity-purified antibodies were used: RBP2 antibody 1416,[39] and JARID1B (KDM5B) antibody BL3841 (Bethyl Laboratories, Inc.). The rabbit antiserum 4440 raised against a GST-PLU1 polypeptide was used as a complementary KDM5B antibody. ChIP experiments in MEFs were performed

using a different KDM5B antibody (antiserum 4440) from the experiments in SAOS-2 cells (where the antibody BL3841 was used), thus, suggesting that the binding of KDM5B antibodies is specific. Primers for real-time PCR were designed in the KDM5A bound regions[13] which were present on the human 19K array[53] or demonstrated high enrichment in ChIPseq experiments in mouse ES cells (data not shown). The sequences of primers for real-time PCR are given in the **Supplemental Table**. The sequences for the control intergenic region INT26D were listed previously.[13] ChIP-qPCR signals were presented as % of input.

Binding sites distribution analysis. The distribution of binding sites and the distance from the TSS for the different HMT and HDM (including JARID2) was calculated using the Bioconductor package ChIPpeakAnno[94] for Ensembl genes (for human version 54 and for mouse version 55), using the peak coordinates from the original publications. Overlap of the peak with the TSS is considered if an overlap of at least one base is found. The distance from the TSS was calculated as the distance of the peak start site from the TSS.

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Note

Supplemental materials can be found at: www.landesbioscience.com/journals/cc/article/14705

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