PRMT6-mediated methylation of R2 in histone H3 antagonizes H3 K4 trimethylation

Dawin Hyllus,^{1,7} Claudia Stein,^{1,7} Kristin Schnabel,² Emile Schiltz,³ Axel Imhof,⁴ Yali Dou,⁵ James Hsieh,⁶ and Uta-Maria Bauer^{1,8}

¹Institute of Molecular Biology and Tumor Research (IMT), Philipps-University of Marburg, 35032 Marburg, Germany;

²Biotechnological Center, Technical University of Dresden, 01307 Dresden, Germany; ³Institute of Organical Chemistry and Biochemistry, Albert-Ludwigs-University Freiburg, 79104 Freiburg, Germany; ⁴Central Laboratory of Protein Analysis, Adolf Butenandt Institute, University of München, 80336 München, Germany; ⁵Department of Pathology, Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48109, USA; ⁶Molecular Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, USA

The arginine methyltransferase PRMT6 (protein arginine methyltransferase 6) has been shown recently to regulate DNA repair and gene expression. As arginine methylation of histones is an important mechanism in transcriptional regulation, we asked whether PRMT6 possesses activity toward histones. We show here that PRMT6 methylates histone H3 at R2 and histones H4/H2A at R3 *in vitro*. Overexpression and knockdown analysis identify PRMT6 as the major H3 R2 methyltransferase *in vivo*. We find that H3 R2 methylation inhibits H3 K4 trimethylation and recruitment of WDR5, a subunit of the MLL (mixed lineage leukemia) K4 methyltransferase complex, to histone H3 *in vitro*. Upon PRMT6 overexpression, transcription of Hox genes and Myc-dependent genes, both well-known targets of H3 K4 trimethylation, decreases. This transcriptional repression coincides with enhanced occurrence of H3 R2 methylation and PRMT6 as well as reduced levels of H3 K4 trimethylation and MLL1/WDR5 recruitment at the HoxA2 gene. Upon retinoic acid-induced transcriptional activation of HoxA2 in a cell model of neuronal differentiation, PRMT6 recruitment and H3 R2 methylation are diminished and H3 K4 trimethylation increases at the gene. Our findings identify PRMT6 as the mammalian methyltransferase for H3 R2 and establish the enzyme as a crucial negative regulator of H3 K4 trimethylation and transcriptional activation.

[**Keywords:** Arginine methylation; PRMT6; histone H3 R2 methylation; histone H3 K4 trimethylation; MLL; transcriptional regulation]

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Covalent post-translational modifications of histone N termini—like methylation, acetylation, or phosphorylation—fulfill fundamental functions in the regulation of gene expression and in the transcriptional memory during cell division (Strahl and Allis 2000; Turner 2002). Depending on the nature and specific site of modifications, these histone marks determine either an active or repressed chromatin state (Jenuwein and Allis 2001). Recent genome-wide profiling of methylation patterns of lysine residues in histones, which can be mono-, di-, or trimethylated, revealed that di- and trimethylation of H3 K27 and K9 associate with silenced genes and heterochromatin (Martens et al. 2005; Boyer et al. 2006; Roh et

al. 2006; Barski et al. 2007). In contrast, H3 K4 di- and trimethylation are found in the 5' region of transcribed genes and correlate with histone acetylation and occupancy of active RNA polymerase (Schubeler et al. 2004; Bernstein et al. 2005; Barski et al. 2007).

Several H3 K4 methyltransferases have been identified in mammals, among them the MLL (mixed lineage leukemia) protein family, Set1, Set7/9, and Ash1 (Ruthenburg et al. 2007). Similar to other histone-modifying enzymes, lysine methyltransferases occur in multiprotein complexes; for example, MLL proteins associate with WDR5, RbBP5, and Ash2 to assemble the core MLL complex (Nakamura et al. 2002; Hughes et al. 2004; Dou et al. 2005). The WDR5 subunit recognizes via its WD40 domain the first three amino acids of the H3 N terminus and in this way presents the K4 side chain for methylation to the catalytically active subunit MLL (Couture et al. 2006; Ruthenburg et al. 2006). H3 recognition by

⁷These authors contributed equally to this work.

⁸Corresponding author.

E-MAIL bauer@imt.uni-marburg.de; FAX 49-6421-2865196.

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WDR5 appears to be tightly regulated, as the presence of adjacent histone modifications, like H3 R2 methylation, abolishes WDR5 interaction in *in vitro* binding assays (Couture et al. 2006). Furthermore, H3 K4 di-/trimethylation and H3 R2 methylation appear to counter correlate in E-box-containing gene promoters (Guccione et al. 2006). Interestingly, an H3 R2 methyltransferase has not yet been identified, neither in mammals nor in other species.

Arginine methylation of histones and other nuclear proteins is performed by the family of PRMTs (protein arginine methyltransferases) that contains nine members in humans (Boisvert et al. 2005; Cook et al. 2006). PRMTs use *S*-adenosylmethionine (SAM)-dependent methylation to modify the guanidino nitrogens of the arginine side chain by adding one or two methyl groups (Bedford and Richard 2005). With regard to the dimethylation product, PRMTs are distinguished into type I enzymes, which catalyze the asymmetric $N^G,N^{G'}$ -dimethyl-arginine, and the type II subfamily, which consists of PRMT5, PRMT7, and PRMT9 and generates symmetric $N^G,N^{G'}$ -dimethylation (Bedford and Richard 2005; Cook et al. 2006). Similar to other histone modifications, histone arginine methylation contributes to transcriptional regulation and is catalyzed by a subset of family members: PRMT1 methylates H4/H2A at R3 (Wang et al. 2001b), PRMT4/CARM1 (coactivator-associated arginine methyltransferase 1) methylates H3 at R17/R26 (Schurter et al. 2001; Bauer et al. 2002), and PRMT5 methylates H3 at R8 and H4/H2A at R3 (Pal et al. 2004). Histone-modifying PRMTs are recruited to the chromatin by their interaction with transcription factors and regulate gene activation as well as repression (Bedford and Richard 2005). The molecular mechanism that allows histone arginine methylation to alter gene expression and chromatin states is still unclear.

Recently, a novel member of the PRMT family, PRMT6, was cloned (Frankel et al. 2002) and shown to be implicated in nucleotide excision repair (El-Andaloussi et al. 2006) and viral transcription (Boulanger et al. 2005). Given that PRMT6 resides predominantly in the nucleus (Frankel et al. 2002), we asked whether it possesses methyltransferase activity toward histones and influences cellular gene expression. Here we identify the human PRMT6 protein as the major H3 R2 methyltransferase *in vivo*, which represses transcription by counteracting H3 K4 trimethylation. Our data establish for the first time the mechanistic consequences of histone arginine methylation and reveal a negative cross-talk between arginine methylation and lysine methylation of histones.

Results

Human PRMT6 is a novel histone arginine methyltransferase

To investigate whether human PRMT6 possesses methyltransferase activity toward histones, we cloned full-length PRMT6 cDNA (Frankel et al. 2002) from MCF7

cells and expressed the protein as GST fusion in *Escherichia coli*. Purified GST-PRMT6 was employed in *in vitro* methylation assays using bulk histones, purified core histones from calf thymus, or recombinant *Drosophila* histones H3 and H4, respectively, in the presence of methyl- ^{14}C -labeled SAM. The GAR (glycine-arginine rich) domain of fibrillarin, an established substrate of PRMT6 (Frankel et al. 2002), served as positive control for the enzymatic activity of the GST-PRMT6 preparation (Fig. 1A). We found free histones H3, H4, and H2A to be predominantly *in vitro* methylated by PRMT6 (Fig. 1A). Furthermore, H2B was also weakly methylated. Reconstituted nucleosomes were no substrate for GST-PRMT6 (Supplementary Fig. S1). Using tailless recombinant histone H3 and H4 incorporated into histone octamers, we mapped the methylation sites of PRMT6 to the N-terminal tails of H3 and H4 (Supplementary Fig. S2). As an arginine residue does not reside in the N-terminal tail (within the first 25 amino acids) of H2B, we assumed that methylation by PRMT6 occurs here close to or within the histone fold or C terminus. To determine the exact arginine residue(s) of PRMT6 methylation in the three other core histones, we performed Edman degradation of calf thymus H3 and of recombinant H4 and H2A that had been labeled with methyl- ^3H SAM and recombinant PRMT6. In this way, we identified Arg 2 (R2) in histone H3 and Arg 3 (R3) in histones H4 and H2A as highly specific methylation sites of PRMT6 (Fig. 1B). The radioactivity remaining on the PVDF membrane after 25 or 30 cycles of Edman degradation indicated that PRMT6 does not further methylate arginines in the histone fold or the C terminus of H3 and H4, but possibly of H2A (data not shown).

Some of the arginines targeted by PRMT6 overlap with the substrate specificity of other PRMTs, as R3 in histone H4 is modified by PRMT1 (Wang et al. 2001b; Wagner et al. 2006) and PRMT5 (Fabrizio et al. 2002; Pal et al. 2004). For R2 in histone H3, it was recently suggested that PRMT4 also exerts *in vitro* weak activity toward this residue (Schurter et al. 2001). To distinguish between unique and common methylation sites of PRMT6 in histone H3 and H4 we performed *in vitro* methylation assays followed by autoradiography as well as Western blot analysis using site-specific methyl-arginine antibodies. PRMT4 and PRMT6 revealed equally strong methylation activity toward histone H3 in the autoradiography, but exclusively the methylation product of PRMT6 contained H3 R2 asymmetric dimethylation (Fig. 1C). In contrast, H4 R3 methylation was detected equally well in PRMT1 and PRMT6 methylation reactions (Fig. 1D). These results suggest that the R2 residue in H3 is predominantly targeted by PRMT6 and not by PRMT4, whereas R3 in H4 is a common site of several PRMTs.

R2 in histone H3 is the major methylation site of PRMT6 in histones in vivo

To investigate whether histone arginine methylation is also catalyzed by PRMT6 *in vivo*, we established MCF7 cell clones stably overexpressing TAP-tagged PRMT6

Figure 1. Human PRMT6 methylates histone N termini in vitro. (A) Bulk histones from calf thymus, purified core histones from calf thymus, recombinant *Drosophila* histones H3 and H4, and, as positive control, GST-GAR were subjected to in vitro methylation in the presence of purified GST-PRMT6 and ^{14}C -labeled SAM. Methylation products were resolved on SDS-PAGE, blotted, and visualized by autoradiography (overnight exposure). Corresponding Coomassie Blue-stained SDS gel visualizes the histones used in the methylation assay. GST-GAR served as a positive control for PRMT6 activity. (B) Purified histone H3 from calf thymus and recombinant *Drosophila* histones H4 and H2A were methylated in vitro in the presence of purified GST-PRMT6 and ^3H -labeled SAM and subsequently subjected to Edman degradation of residues 1–30 for H3 and H4 and residues 1–25 for H2A. Phenylthiohydantoin amino acid fractions were analyzed for the presence of tritium by scintillation counting. (C,D) Recombinant *Drosophila* histone H3 (C) or H4 (D) was methylated in the presence of the indicated GST-PRMTs and ^{14}C -labeled SAM. Methylation reactions were separated by SDS-PAGE and blotted. Blots were analyzed by autoradiography and by Western blot with the indicated antibodies (anti-H3 R2me2 in C, and anti-H4 R3me2 in D). The corresponding Coomassie stains are shown.

protein. The endogenous and exogenous PRMT6 expression levels of two control MCF7 cell clones (nos. 1 and 5) and two PRMT6-overexpressing clones (nos. 11 and 18) are presented in Figure 2A. Overexpression of PRMT6 in these cell clones resulted in a globally increased level of asymmetric dimethylated R2 in H3 compared with control cells, as shown by Western blot (Fig. 2B) and immunofluorescence staining (Fig. 2C). Concordant results were also obtained with a transient overexpression of PRMT6, whereas a methylation-deficient mutant of PRMT6 did not cause enhanced H3 R2 dimethylation (Supplementary Fig. S3). The methylation levels of H4 R3 (Fig. 2D) and H3 R17 (Supplementary Fig. S4) remained unchanged in the presence of exogenous PRMT6. Conversely, transient transfection of short interfering RNA (siRNA) directed against the PRMT6 transcript in HEK293 cells resulted in the depletion of the endogenous PRMT6 protein and correlated with reduced levels of H3 R2 dimethylation in comparison with control short interfering green fluorescent protein (siGFP)-transfected cells (Fig. 2E). These results indicate that PRMT6 predominantly asymmetrically dimethylates R2 in histone H3 in vivo and identify the enzyme as the major cellular H3 R2 methyltransferase.

PRMT6 preferentially catalyzes the addition of the second methyl group onto monomethyl R2 in H3

Given that PRMTs catalyze mono- as well as dimethylation of arginine residues, we analyzed the enzymatic properties of PRMT6 by using as substrates histone H3 peptides either unmodified, monomethylated (R2me1), or asymmetrically dimethylated (R2me2) at R2 in a methyltransferase assay. We found that PRMT6 methylates unmodified H3 peptide, but shows enhanced activity toward the H3 R2me1 peptide (Fig. 2F), suggesting that the enzyme preferentially catalyzes the addition of the second methyl group onto monomethyl R2. In agreement with this, mass-spectrometric analysis of PRMT6-methylated H3 peptides showed that the unmodified H3 peptide used as substrate was weakly monomethylated at R2 and not dimethylated (Supplementary Fig. S5). The premodified H3 R2me1 substrate revealed strong dimethylation at R2 subsequent to in vitro methylation by PRMT6, whereas the premodified H3 R2me2 peptide served as negative control (Supplementary Fig. S5). In agreement with this observation, monomethylation levels of H3 R2 remained unchanged in the presence of exogenous PRMT6 (Supplementary Fig. S6).

Figure 2. PRMT6 methylates R2 in histone H3 in vivo. (A) Fifty micrograms of whole-cell extracts of MCF7 clones, either overexpressing PRMT6 (clone nos. 11 and 18) or control (clone nos. 1 and 5), were analyzed by Western blot using antibodies against human PRMT6 for their endogenous (asterisk) and exogenous (arrow) PRMT6 expression levels. The Western blot for β -tubulin served as loading control. (B) Twenty-five micrograms of extracts of PRMT6-overexpressing MCF7 clones (nos. 11 and 18) and control clones (nos. 1 and 5) were analyzed by Western blot for the level of histone H3 R2 dimethylation (anti-H3 R2me2). The Western blot for histone H3 served as loading control. (C) PRMT6-overexpressing MCF7 clone (no. 11) and the control clone (no. 5) were analyzed by immunofluorescence staining for PRMT6 and histone H3 R2 dimethylation (anti-H3 R2me2). The corresponding DNA stainings with DAPI are shown on the right. Images of the overexpressing and control cells were taken with the same exposure times. (D) Thirty micrograms of extracts of PRMT6-overexpressing MCF7 clones (nos. 11 and 18) and control clones (nos. 1 and 5) were analyzed by Western blot for levels of histone H4 R3 dimethylation (anti-H4 R3me2). The Western blot for histone H3 served as loading control. (E) HEK293 cells were transfected with siRNAs against PRMT6 (siPRMT6) and GFP (siGFP) and harvested 48 h post-transfection. Fifteen micrograms of extracts were analyzed by Western blot for PRMT6 and 30 μ g of extracts were analyzed by Western blot for histone H3 R2 dimethylation (anti-H3 R2me2). Western blots for histone H3 and β -tubulin served as loading controls. (F) Histone H3 peptides (unmodified, R2me1, and R2me2) were subjected to in vitro methylation in the presence of GST-PRMT6 and 3 H-labeled SAM. Methylation reactions were separated by SDS-PAGE and blotted. Membrane pieces with the radiolabelled peptides were quantified by scintillation counting. A methylation assay without peptide substrate (PRMT6 alone) served as control.

H3 R2 methylation of PRMT6 interferes with H3 K4 trimethylation and WDR5 binding

As recent studies provided evidence of a potential negative regulation of H3 K4 di-/trimethylation by H3 R2 methylation (Couture et al. 2006; Guccione et al. 2006), we investigated whether PRMT6 could be the enzyme responsible for such an antagonism. Using the MCF7 cell clones stably overexpressing PRMT6, we found that H3 K4 trimethylation is globally decreased in the presence of exogenous PRMT6 (Fig. 3A,B; Supplementary Fig. S7). We did not observe such a global change in H3 K4 trimethylation when we transiently overexpressed PRMT6 (data not shown). Conversely, siRNA-mediated knockdown of endogenous PRMT6 caused an increase in the level of H3 K4 trimethylation in comparison with control siGFP-transfected cells (Fig. 3C). To exclude that the observed antagonism between R2 dimethylation and K4

trimethylation is due to antibody epitope occlusion, we confirmed by Western blot analysis that the antibodies employed here against the two modifications are able to recognize a double-modified (R2me2/K4me3) H3 peptide (Supplementary Fig. S8). These results indicate that the H3 R2 methyltransferase identified here, PRMT6, is involved in the regulation of the H3 K4 trimethylation state of chromatin in vivo.

To determine by which mechanism PRMT6 and H3 R2 methylation influence H3 K4 trimethylation, we performed in vitro methylation assays with unmodified and premodified H3 peptides using recombinant K4 methyltransferases MLL1 (Milne et al. 2002; Nakamura et al. 2002), MLL1 complex affinity-purified via Flag-tagged WDR5 (Dou et al. 2005), and recombinant Set7/9 (Wang et al. 2001a; Nishioka et al. 2002). The activity of recombinant MLL1 was diminished toward H3 R2me1 peptide and strongly inhibited toward H3 R2me2 peptide in com-

Figure 3. PRMT6 and H3 R2 methylation interferes with H3 K4 trimethylation and binding of WDR5 to the H3 N terminus. (A,B) PRMT6-overexpressing MCF7 clones (nos. 11 and 18) and the control clones (nos. 1 and 5) were analyzed by immunofluorescence staining for histone H3 K4 trimethylation (anti-H3 K4me3). The corresponding DNA stainings with DAPI are shown on the right. Images of the overexpressing and control cells were taken with the same exposure times. (C) HEK293 cells were transfected with siRNAs against PRMT6 (siPRMT6) and GFP (siGFP as control). Forty-eight hours post-transfection, cells were harvested and 30 μ g of extracts were analyzed by Western blot for histone H3 K4 trimethylation (anti-H3 K4me3). The Western blot for histone H3 served as loading control. (D,E) The indicated histone H3 peptides were methylated in the presence of 3 H-labeled SAM and GST-MLL1 (amino acids 3745–3969) (D) or purified MLL1 complex (E). Methylation reactions were separated by SDS-PAGE and blotted. Membrane pieces with the radiolabeled peptides were quantified by scintillation counting. Methylation assays without peptide substrate served as controls. (F) The indicated histone H3 peptides were methylated in the presence of His-Set7/9 and 3 H-labeled SAM. Methylation reactions were spotted on P81 paper and were quantified by scintillation counting. A methylation assay without peptide substrate served as control. (G) The indicated histone H3 peptides were

coupled to beads and incubated with HEK293 whole-cell extract overexpressing Flag-tagged WDR5. Bound proteins and 10% extract input (10 μ g) were resolved by SDS-PAGE and analyzed by anti-Flag Western blot for the presence of WDR5. Silver staining of the H3 peptides served as loading control.

parison with its activity on the unmodified H3 peptide (Fig. 3D). Similarly, the activity of the MLL1 complex was reduced toward the H3 R2me2 peptide (Fig. 3E). Set7/9 was not impaired in its K4 methyltransferase activity by H3 R2 methylation (Fig. 3F). H3 peptides pre-methylated at K4 did not serve as substrate for MLL1 and Set7/9 (Fig. 3D,F), confirming the specificity of both methyltransferases. These results suggest that some K4 methyltransferases, like MLL1, are affected in their catalytic activity by pre-methylation of R2. Furthermore, we investigated whether the methyltransferase activity of PRMT6 is influenced by pre-existing histone H3 modifications. We found that PRMT6 activity toward H3 peptides is reduced in the presence of H3 K4 or H3 K9 di-/trimethylation and slightly enhanced in the presence of H3 K27 di-/trimethylation (Supplementary Fig. S9).

Given that MLL1 is part of a multiprotein complex, in which the WDR5 subunit is responsible for the recognition of the H3 tail (Wysocka et al. 2005), we analyzed whether WDR5 binding to H3 is influenced by R2 methylation. Peptide pull-down experiments with extracts from Flag-tagged WDR5-overexpressing cells revealed a strong binding of WDR5 to unmodified H3 peptide as

well as H3 R2me1 peptide, whereas WDR5 interaction was completely abolished in the presence of H3 R2me2 (Fig. 3G). These findings suggest that binding of WDR5 to the H3 N terminus and the catalytic activity of the WDR5-containing MLL1 complex are regulated by H3 R2 methylation.

PRMT6 inhibits transcription of genes known to be regulated by H3 K4 trimethylation

Our results suggest that increased global levels of H3 R2 methylation caused by PRMT6 overexpression might inhibit recruitment of WDR5 as well as methyltransferase activity of MLL1, and result accordingly in a global decrease of H3 K4 trimethylation. Such events should have an impact on the transcriptional activation of genes that are regulated by the MLL complex. Well-known targets of MLL1 and H3 K4 trimethylation are Hox genes (Milne et al. 2002; Nakamura et al. 2002; Hughes et al. 2004). Therefore, we investigated the transcription levels of HoxA genes in the MCF7 cell lines stably overexpressing PRMT6 versus control cell lines. Overexpression of

Figure 4. PRMT6 overexpression inhibits Hox gene transcription and results in a decrease of H3 K4 trimethylation and MLL1/WDR5 recruitment at the HoxA2 gene locus. (A,B) Total RNA was prepared from PRMT6-overexpressing MCF7 cells (clone nos. 11 and 18) and control cells (clone nos. 5 and 1) analyzed by RT-QPCR for transcript levels of PRMT6 (A) and the genes HoxA2, HoxA10, HoxA5, and HoxA6 (B) normalized for GAPDH. The PRMT6-overexpressing MCF7 clones are illustrated in black bars (no. 11) and dark gray bars (no. 18), and the control clones in white bars (no. 5) and light gray bars (no. 1). (C,D) PRMT6-overexpressing MCF7 cells (clone no. 11) and control cells (clone no. 5) were subjected to ChIP analysis using PRMT6-specific, H3 R2me2-specific, and H3 K4me3-specific antibodies (C) and MLL1-C-specific (C terminus), MLL1-N-specific (N terminus), and WDR5-specific antibodies (D). IgG and preimmune serum of anti-PRMT6 were employed as control antibodies. Immunoprecipitated DNA was analyzed in triplicate by QPCR with primers for the HoxA2 gene (5' and 3' ends of the transcribed region) and the HoxA5 gene (5' end of the transcribed region). Mean values are expressed as percentage input (% input).

PRMT6 was confirmed by RT-quantitative PCR (QPCR) (Fig. 4A). We found that the transcription levels of HoxA2 and HoxA10 are down-regulated in PRMT6-overexpressing cell clones compared with control cell clones (Fig. 4B). This was no global effect on all genes of the HoxA locus, as transcription of HoxA5 and HoxA6 was not influenced (Fig. 4B). Similar results on HoxA gene expression were gained with transient overexpression of PRMT6, whereas a methylation-deficient mutant of PRMT6 revealed no repressive influence on HoxA2 gene transcription (Supplementary Fig. S10). Furthermore, we studied Myc target genes, for which it was recently shown that Myc binding and transcriptional activation correlate with the presence of H3 K4 trimethylation and the absence of H3 R2 methylation in the promoter regions (Guccione et al. 2006). We found that transcript levels of the Myc target genes TERT, nucleolin, Golph3, and prothymosin α (PTMA) were decreased in MCF7 cells with PRMT6 overexpression, whereas the Myc targets LDHA and ODC did not show this effect (Supplementary Fig. S11).

PRMT6 regulates the H3 R2 methylation level, the H3 K4 trimethylation level, and MLL1/WDR5 binding at the HoxA2 gene

In the following section, we investigated whether the transcriptional effects subsequent to PRMT6 overexpression are due to a direct influence of the enzyme on the level of chromatin modifications and coregulator recruitment at the target gene. Therefore, we performed chromatin immunoprecipitation (ChIP) analysis in PRMT6-overexpressing MCF7 cells versus control cells for the enrichment of the HoxA2 gene locus using antibodies for PRMT6, H3 R2 asymmetric dimethylation, and H3 K4 trimethylation. In comparison with control antibodies, we found weak binding of endogenous PRMT6 at the 5' end of the HoxA2 gene close to the transcriptional start site (TSS) in control cells. This PRMT6 recruitment was strongly enhanced upon PRMT6 overexpression (Fig. 4C). In agreement with the catalytic activity of PRMT6, increased levels of H3 R2 dimethylation coincided with this enhanced occurrence of PRMT6, whereas H3 K4 tri-

methylation was reduced at the 5' end of the *HoxA2* gene in PRMT6-overexpressing cells when compared with control cells. In contrast, we did not observe such changes in the recruitment of PRMT6, the levels of H3 R2 dimethylation or H3 K4 trimethylation upon PRMT6 overexpression at the 3' end of the *HoxA2* gene, or around the TSS/5' end of the *HoxA5* gene, a gene transcriptionally not affected by exogenous PRMT6 in MCF7 cells (Fig. 4B,C). Consistent with this gene-specific reduction of K4 trimethylation upon PRMT6 overexpression and our *in vitro* data on impaired recognition of R2 dimethylated H3 peptide by WDR5, binding of MLL1 and WDR5 was diminished at the 5' end of the *HoxA2* gene in PRMT6-overexpressing cells (Fig. 4D). In contrast, no clear reduction of MLL1 binding and even enhanced WDR5 binding was observed at the 3' end of *HoxA2* or at the TSS of *HoxA5* in the presence of exogenous PRMT6 (Fig. 4D). These results indicate that PRMT6-mediated H3 R2 dimethylation influences *in vivo* recruitment of MLL1 and WDR5 to chromatin and H3 K4 trimethylation at the gene level.

Finally, we analyzed in a cell model of neuronal differentiation, NT2/D1 cells (Andrews 1984), whether in ChIP analysis recruitment of endogenous PRMT6 correlates with *HoxA2* gene repression and whether PRMT6 is released upon transcriptional induction. In NT2/D1 cells, a subset of *HoxA* genes—among them *HoxA2*—has recently been shown to be transcriptionally inducible upon all-*trans*-retinoic acid (ATRA) treatment (Bracken et al. 2007). As shown in Figure 5A, the *HoxA2* gene is transcriptionally activated upon stimulation of NT2/D1

cells with ATRA. ChIP analysis revealed that PRMT6 recruitment and H3 R2 methylation at the *HoxA2* gene is strongest in unstimulated/undifferentiated cells and gradually reduces during the time course of ATRA treatment. Conversely, H3 K4 trimethylation strongly increased at the *HoxA2* gene in the course of ATRA-induced neuronal differentiation, coinciding with transcriptional activation and loss of H3 R2 methylation (Fig. 5B). As a control, we investigated PRMT6 binding and the two H3 modifications at the *GAPDH* gene and found no substantial changes during the ATRA time course. These results suggest that PRMT6 antagonizes H3 K4 trimethylation and transcriptional activation via its catalytic activity toward R2 in H3.

Discussion

In the present work, we identified human PRMT6 as a novel histone arginine methyltransferase and showed that the enzyme methylates all four core histones *in vitro*, predominantly H3, H4, and H2A. Radiosequencing of the N-terminal histone tails revealed that R2 in histone H3 and R3 in histones H4 and H2A are the major sites for PRMT6-mediated methylation. Given that the first five N-terminal amino acids (SGRGK) are identical in H4 and H2A, it seems evident that R3 is methylated in both histone tails by PRMT6. Similar observations have also been made for PRMT1 and PRMT5, both of which modify R3 in H4 and H2A (Wang et al. 2001b; Ancelin et al. 2006). Some of the arginines targeted by PRMT6 in histones overlap with the substrate specific-

Figure 5. Binding of endogenous PRMT6 to the *HoxA2* gene coincides with transcriptional repression and is released upon transcriptional activation of the gene. (A) NT2/D1 cells were left untreated or were treated for 3 and 12 d with 1 μ M ATRA. Subsequently, total RNA was prepared and analyzed by RT-QPCR for transcript levels of *HoxA2* normalized for *GAPDH*. (B) NT2/D1 cells either undifferentiated (without ATRA) or differentiated for 3 and 12 d with ATRA, respectively, were subjected to ChIP analysis using PRMT6-specific, H3 R2me2-specific, and H3 K4me3-specific antibodies. IgG was employed as control antibody. Immunoprecipitated DNA was analyzed in triplicate by QPCR with primers for the *HoxA2* gene (5' end) and the *GAPDH* gene. Mean values were expressed as fold enrichment compared with IgG. (C) Model for the antagonism between PRMT6-catalyzed histone H3 R2 methylation and recruitment of the K4 methyltransferase complex and H3 K4 trimethylation. PRMT6 is bound to a subset of

transcriptionally inactive *Hox* genes and presumably also *Myc*-dependent genes and catalyzes dimethylation of R2 in histone H3 at the 5' end (near the TSS) of the target genes. In doing so, PRMT6 on the one hand directly blocks the catalytic activity of the H3 K4 methyltransferase MLL1, and on the other hand abolishes recruitment of WDR5 and other subunits of the WDR5-containing methyltransferase complex—e.g., MLL1—that collectively lead to transcriptional repression by inhibition of H3 K4 trimethylation. In the course of transcriptional induction, PRMT6 detaches from its target genes and in this way allows H3 K4 trimethylation, followed by active transcription.

ity of other PRMTs. For example, it was shown that R3 in H4 is modified by PRMT1 in the context of coactivation of nuclear receptor-regulated transcription (Wang et al. 2001b; Wagner et al. 2006), as well as by PRMT5 during repression of E2F4 and Myc target genes (Fabbri et al. 2002; Pal et al. 2004). Likewise, for R2 in H3 it was recently suggested that PRMT4, which predominantly methylates R17 in H3 (Ma et al. 2001; Bauer et al. 2002), exerts activity toward R2 (Schurter et al. 2001; Torres-Padilla et al. 2007). However, here we show that R2 in H3 seems to be a unique site for asymmetric dimethylation by PRMT6. In contrast, R3 in H4 was equally well methylated by PRMT1 and PRMT6 and therefore presents a common site of both enzymes. Using overexpression and depletion analysis of PRMT6, we found that the enzyme also targets *in vivo* mainly R2 in histone H3 on a global level, whereas the level of H4 R3 methylation or other histone arginine methylation sites, like H3 R17, remained unaffected by PRMT6, in agreement with our *in vitro* findings.

By studying the enzymatic properties of PRMT6, we discovered that it possesses a distinct substrate specificity from other PRMT family members. So far most PRMTs have been reported to perform dimethylation of unmodified substrates, indicating that they efficiently carry out monomethylation and subsequent dimethylation (Bedford and Richard 2005). In contrast, PRMT6 preferentially catalyzed in *in vitro* assays the dimethylation step of H3 peptide monomethylated at R2. These results were supported by the *in vivo* observation showing that PRMT6 overexpression did not alter the global monomethylation levels of H3 R2. Previously, PRMT7 was suggested to preferentially perform monomethylation (Miranda et al. 2004), implying that there might indeed exist arginine methyltransferases that mainly monomethylate arginine residues and that could act as prerequisites or enhancers for enzymes catalyzing the subsequent dimethylation step, such as PRMT6.

As recent studies suggested a negative cross-talk between H3 K4 di-/trimethylation and H3 R2 methylation (Couture et al. 2006; Guccione et al. 2006), we investigated whether PRMT6 could be the enzyme responsible for such an antagonism. We found that PRMT6-mediated H3 R2 dimethylation compromises the enzymatic activity of the MLL1 K4 methyltransferase and inhibits global H3 K4 trimethylation. Additionally, binding of WDR5 to the H3 R2me₂ peptide was abolished when compared with its interaction with the unmodified H3 peptide and the H3 R2me₁ peptide. These findings agree with recent data showing that binding of recombinant WDR5 to H3 peptides is not detectable in the presence of R2 methylation or R2A mutation (Couture et al. 2006). Furthermore, the crystal structure analysis of WDR5 revealed extensive hydrogen bonding between its WD40 domain and the guanidino nitrogens of R2, and suggested that WDR5 presents the K4 side chain for methylation to the catalytic active subunit MLL (Couture et al. 2006; Ruthenburg et al. 2006). Interestingly, a mutation in WDR5 (Y191A) that reduces its binding to H3 R2 was reported to lead to a significant loss of the histone meth-

yltransferase activity of the MLL complex, indicating that WDR5 binding to H3 affects MLL activity (Dou et al. 2006). As WDR5 is also part of the Set1 complex, which possesses methyltransferase activity toward H3 K4 (Wysocka et al. 2003), it seems likely that other K4 methyltransferases are regulated in their catalytic activity and substrate recognition by H3 R2 methylation as well. However, our results suggest that not all H3 K4 methyltransferases—for example, Set7/9—are affected in their activity by premethylation of R2. Such differences might be due to distinct mechanisms in substrate recognition and catalysis of the various H3 K4 methyltransferases, as, for example, Set7/9 acts as a monomeric enzyme and exclusively monomethylates K4 (Wang et al. 2001a; Nishioka et al. 2002; Xiao et al. 2003). In contrast, MLL1 is part of a larger protein complex containing WDR5 and performs mono-, di-, and trimethylation of K4 (Dou et al. 2005). Hence, our data reveal that H3 R2 dimethylation has a dual function: It regulates the binding of WDR5 to the H3 N terminus and the catalytic activity of the WDR5-containing MLL1 complex, the latter either directly or indirectly via WDR5.

Interestingly, we also found that PRMT6 activity toward the H3 peptide is altered by pre-existing modifications; for example, the presence of H3 K4 or H3 K9 di-/trimethylation causes a reduction of its activity, and the presence of H3 K27 di-/trimethylation causes an enhancement of the PRMT6 activity. These data imply that R2 methylation is not only inhibitory for K4 methylation but also vice versa, supporting the antagonism of both modifications. H3 K27 trimethylation was shown to correlate with the appearance of H3 R2 methylation (Guccione et al. 2006), which might be due to the positive cross-talk observed here.

The negative regulation of the H3 K4 trimethylation levels by PRMT6-mediated H3 R2 dimethylation should have an impact on the transcriptional activity of genes that are regulated by the MLL complex. For Hox genes and Myc-dependent genes, both of which are well-known targets of MLL and H3 K4 trimethylation (Milne et al. 2002; Nakamura et al. 2002; Hughes et al. 2004), we found here that the transcript level of a subset of these genes is negatively influenced by PRMT6. Although we found that PRMT6 overexpression has a strong effect on the global level of H3 R2 methylation, PRMT6 seems to repress transcription via its catalytic activity toward R2 in H3 rather gene specifically; for example, HoxA2 and HoxA10 transcription were decreased upon PRMT6 overexpression, whereas HoxA5 and HoxA6 remained unaffected. ChIP analysis revealed that the transcriptional effects of PRMT6 are conducted by gene-specific recruitment of the enzyme to the chromatin, which was accompanied by enhanced H3 R2 methylation. These events coincided with a decreased level of H3 K4 trimethylation and loss of WDR5 and MLL1 binding to the HoxA2 gene, indicating that PRMT6-mediated H3 R2 dimethylation also antagonizes *in vivo* recruitment of WDR5 to chromatin and the function of the MLL1 complex. As several reports suggested that MLL1 recruitment does not require the presence of WDR5 (Wysocka

et al. 2005, 2006; Dou et al. 2006), our observation implies that R2 methylation might directly or indirectly—for example, as suggested above, via the WDR5 subunit or other subunits of the complex—interfere with the binding affinity of MLL1 to chromatin.

Furthermore, we found in a cell model of neuronal differentiation, in which HoxA2 gene expression is transcriptionally silenced in the undifferentiated state and becomes activated in the course of ATRA-induced differentiation (Bracken et al. 2007), that endogenous PRMT6 is bound to the transcriptionally inactive HoxA2 gene and H3 R2 methylation levels are high. Coinciding with neuronal differentiation and transcriptional activation of HoxA2, PRMT6 and H3 R2 dimethylation disappeared from the target gene promoter and H3 K4 trimethylation strongly increased. These data underline the dynamic nature of the transcriptional repression mechanism by PRMT6 described here and summarized in the model in Figure 5C. As the repressive function of PRMT6 is gene-specific and seems not to globally affect gene expression regulated by MLL, the regulation of the H3 K4 trimethylation state reported here might be an alternative mechanism. Together with the recently discovered H3 K4 demethylases, there might exist several gene-specific mechanisms for regulation of H3 K4 trimethylation levels in the cell (Shi et al. 2004; Lee et al. 2005; Klose et al. 2007).

In summary, these findings identify PRMT6 as the major cellular H3 R2 dimethyltransferase and an important negative regulator of a subset of genes that are activated by H3 K4 trimethylation. PRMT6 fulfills this function by antagonizing chromatin recruitment of WDR5 as well as MLL1 of the K4 methyltransferase complex, and by inhibition of the catalytic activity of MLL1. This antagonism elucidates for the first time a detailed molecular consequence of histone arginine methylation and reveals a negative cross-talk between arginine methylation and lysine methylation of histones.

Materials and methods

Cell lines and antibodies

HEK293, MCF7, and NT2/D1 cells were maintained in DMEM supplemented with 10% fetal calf serum (FCS, GIBCO-BRL) at 37°C and 5% CO₂. To induce neuronal differentiation of NT2/D1 cells with 1 μM ATRA (Sigma), we followed the protocols of Andrews (1984) and Bracken et al. (2007). The following antibodies were employed: anti-PRMT6 and corresponding preimmune serum (Wagner et al. 2006), anti-H3 from Upstate Biotechnology (05-499), anti-H3 R2me2 (asymmetric) from Upstate Biotechnology (07-585) for Western blot/ChIP and from Abcam (ab8046) for immunofluorescence, anti-H4 R3me2 (asymmetric) from Upstate Biotechnology (07-213), anti-H3 K4me3 from Abcam (ab8580) for immunofluorescence and from Cell Signaling Technology (9751); for Western blot/ChIP, anti-Flag from Sigma (F3165), anti-β-tubulin from Chemicon (MAB3408), and rabbit IgG from Sigma. The WDR5 antibody (Wysocka et al. 2003) was a kind gift of W. Herr. The MLL1 antibodies (against the N terminus and C terminus, respectively) were published by Hsieh et al. (2003) and Takeda et al. (2006).

Cloning and plasmids

The ORF sequence of human PRMT6 (GenBank accession no. AY043278) was PCR-amplified with a 5' BamHI site-introducing primer and a 3' EcoRI site-introducing primer using cDNA prepared from MCF7 cells and cloned into pGEX-2TK (GE Healthcare Life Science) and pNTAP B (Stratagene), respectively. The pET28b-hSet7/9 construct was published by Nishioka et al. (2002) and the pGEX-hMLL1/ALL1 Set domain (amino acids 3745–3969) was published by Nakamura et al. (2002). The pcDNA3.1/Hygro Flag-hWDR5-HA construct was described previously by Lee and Skalnyk (2005), and the pGEX2T-GAR was described by Tang et al. (1998). The pVax-Myc-PRMT6 wt/mut (VLD-KLA) were published by Boulanger et al. (2005).

Transfections of plasmids and siRNAs

MCF7 cells were transfected with pNTAP empty (control) and with the pNTAP-hPRMT6 construct using Eugene 6 (Roche). Stable MCF7 transfectants were selected with 500 μg/mL neomycin (Cayla) 48 h post-transfection. Individual clones were expanded in selection medium and analyzed by Western blot and RT-PCR. Transient plasmid transfection of HEK293 cells was performed using a standard CaPO₄ protocol. siRNA oligonucleotide duplexes were purchased from Dharmacon for targeting the human PRMT6 transcript or the GFP transcript as control siRNA. The siRNA sequences (sense strand indicated) are siPRMT6, 5'-GAGCAAGACACGGACGUUU-3'; and siGFP, 5'-GCAAGCUGACCCUGAAGUU-3'. siRNA duplexes (80 nM final concentration) were transfected in HEK293 cells with Oligofectamine (Invitrogen) for 2 d, and afterward cells were collected for protein extraction.

Extraction of histones from mammalian cells and preparation of recombinant histones

HEK293 and MCF7 cells were lysed in FT lysis buffer (20 mM Tris/HCl at pH 7.8, 600 mM NaCl, 20% glycerol, proteinase inhibitor), and histones were extracted by repeated freeze-thaw cycles. After Benzonase treatment, protein concentration was determined and extracts were analyzed by SDS-PAGE. Recombinant histones and histone octamers were a generous gift of Alexander Brehm and were prepared as described previously (Luger et al. 1997).

Histone methyltransferase assays

GST-PRMT6, GST-GAR, GST-MLL1 (amino acids 3745–3969), and His-Set7/9 were expressed in *E. coli* BL21 and were purified according to standard procedures. Purification of the MLL1–WDR5 complex via Flag-WDR5 was described by (Dou et al. 2005). One to five micrograms of either bulk histones from calf thymus (Sigma), purified core histones from calf thymus (Sigma), or H3 peptides, or 0.1 μg of GST-GAR were incubated with purified His-/GST-tagged methyltransferases or MLL1–WDR5 complex and [¹⁴C-methyl]-SAM (58.3 nCi/mM; GE Healthcare Life Science) or [³H-methyl]-SAM (76 nCi/mM; GE Healthcare Life Science) in PBS for 1–2 h at 30°C. Methyltransferase reactions were analyzed either by SDS-PAGE, blotting, and autoradiography, or scintillation counting. Methylation reactions were performed in triplicate, from which the standard deviation was calculated. Error bars are indicated accordingly.

Edman degradation of radiolabelled histones

Histone H3 from calf thymus and recombinant H4 and H2A were labeled with ³H-SAM using GST-PRMT6 and fractionated

by SDS-PAGE. Proteins were transferred onto PVDF membrane and subjected to Edman degradation. Phenylthiohydantoin amino acid fractions were analyzed by scintillation counting for the presence of ^3H -labeled methyl groups, and in separate sequencer runs, amino acid identity was verified by on-line HPLC.

Synthetic H3 peptides and peptide pull-down

Histone H3 N-terminal peptides encompassing 1–15 and 1–30 amino acids followed by a C-terminal cysteine residue were synthesized by Peptide Specialty Laboratories. Modified peptides were synthesized by using monomethylated arginine, asymmetric dimethylated arginine, dimethylated lysine, or trimethylated lysine as indicated in the figures. Peptides with a C-terminal cysteine residue were coupled to SulfoLink resin (Pierce) according to the manufacturer's protocol. Ten micrograms of synthetic peptide were incubated with 100 μg of HEK293 whole-cell extract (prepared in IPH buffer: 50 mM Tris/HCl at pH 8, 150 mM NaCl, 5 mM EDTA, 0.5% NP40) for 15 min at 4°C. After extensive washes in IPH, buffer-bound proteins were separated by SDS-PAGE and stained by Western blot.

RT and QPCR

Total RNA from MCF7 and NT2/D1 cells was isolated using the peqGold total RNA kit (PeqLab). Two micrograms of RNA were applied to RT by incubation with 0.5 μg of oligo(dT)₁₇ primer and 100 U of M-MLV reverse transcriptase (Invitrogen). cDNA was analyzed by QPCR, which was performed using SybrGreen (Abgene) and the Mx3000P real-time detection system (Stratagene). For RT-QPCR, we used the following primers: hGAPDH: forward, 5'-AGCCACATCGCTCAGACAC-3', and reverse, 5'-GCCCAATACGACCAATCC-5'; hPRMT6: forward, 5'-AGACACGACGTTTCAGGAG-3', and reverse, 5'-CCACTTTGTAGCGCAGCAG-3'; hHoxA2: forward, 5'-TC TGGAGAGGGAAGGCTACA-3', and reverse, 5'-TCGCCAT TGTGTCCATTG-3'; hHoxA10: forward, 5'-CCTTCCGAGAG CAGCAA-3', and reverse, 5'-TTGGCTGCGTTTTCACCT 3'; hHoxA5: forward, 5'-GCGCAAGCTGCACATAAG-3', and reverse, 5'-CGGTTGAAGTGGAATCCTT-3'; and hHoxA6: forward, 5'-ATGCAGCGGATGAACTCC-3', and reverse, 5'-AGGTAGCGGTTGAAGTGGAA-3'. All amplifications were performed in triplicate using 0.5 μL of cDNA per reaction. The triplicate mean values were calculated according to the $\Delta\Delta\text{Ct}$ quantification method (Pfaffl 2001) using the GAPDH gene transcription as reference for normalization. Standard deviation was calculated from the triplicates, and error bars are indicated accordingly. Relative mRNA levels in control MCF7 cells were equated to 1 and the other values were expressed relative to this.

ChIP

NT2/D1 cells, MCF7 control (clone no. 5), and PRMT6-overexpressing (clone no. 11) cells were cross-linked in the presence of 1% formaldehyde for 10 min at 37°C and harvested after washing twice with cold PBS. ChIP was performed as described previously (Wagner et al. 2006). Immunoprecipitated and eluted DNA was purified with QIAquick columns (Qiagen) and were amplified by QPCR with the following primers: HoxA2 gene/5' end of the transcribed region (amplifying nucleotides +107 to +451): forward, 5'-CGGTCCCCATACGGCTGTA-3', and reverse, 5'-CAGGCTGGGAATGGTCTGCT-3'; HoxA2 gene/3' end of the transcribed region (amplifying nucleotides +1952 to +2169): forward, 5'-CAGGTTCCCTCGACAGTCCC-3', and reverse, 5'-AAAGGAGGGAAGGGGTAGGTC-3'; HoxA5 gene/5' end of the transcribed region (amplifying nucleotides -176 to

+16): forward, 5'-CTCCACCCAACCTCCCCTATTAGTG-3', and reverse, 5'-GTGCGTCTATAGCACCTTGC-3'; and GAPDH gene (amplifying nucleotides -353 to -229): forward, 5'-CCATCTCAGTCGTTCCCAAAGTCC-3', and reverse, 5'-GATGGGAGGTGATCGGTGCT-3'. Amplifications were performed in triplicate, and mean values were expressed as percentage input or fold enrichment compared with IgG. Standard deviation was calculated from the triplicates, and error bars are indicated accordingly.

Immunofluorescence staining

MCF7 cells were plated on cover slips. After 24 h, cells were rinsed in PBS and fixed in methanol for 10 min at -20°C. Subsequently cells were permeabilized in PBS/0.2% Triton X-100 and then stained with the indicated antibodies in the presences of PBS/3% BSA for 2 h at room temperature. Afterward, cells were rinsed three times in PBS and stained with the secondary antibody, anti-rabbit Cy3 (Sigma). For nuclear/DNA staining, cells were incubated with 0.1 mg/mL DAPI (4',6-diamidino-2'-phenylindole dihydrochloride) in PBS for 5 min at room temperature. After the final washes in PBS, cells were mounted (Mowiol containing 25 mg/mL DAPCO) and analyzed by fluorescence microscopy (Axioskop 2, Zeiss).

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References

- Ancelin, K., Lange, U.C., Hajkova, P., Schneider, R., Bannister, A.J., Kouzarides, T., and Surani, M.A. 2006. Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nat. Cell Biol.* **8**: 623–630.
- Andrews, P.W. 1984. Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. *Dev. Biol.* **103**: 285–293.
- Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* **129**: 823–837.
- Bauer, U.M., Daujat, S., Nielsen, S.J., Nightingale, K., and Kouzarides, T. 2002. Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO Rep.* **3**: 39–44.
- Bedford, M.T. and Richard, S. 2005. Arginine methylation an

- emerging regulator of protein function. *Mol. Cell* **18**: 263–272.
- Bernstein, B.E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D.K., Huebert, D.J., McMahon, S., Karlsson, E.K., Kulbokas III, E.J., Gingeras, T.R., et al. 2005. Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* **120**: 169–181.
- Boisvert, F.M., Chenard, C.A., and Richard, S. 2005. Protein interfaces in signaling regulated by arginine methylation. *Sci. STKE* **2005**: re2. doi: 10.1126/stke.2712005re2.
- Boulanger, M.C., Liang, C., Russell, R.S., Lin, R., Bedford, M.T., Wainberg, M.A., and Richard, S. 2005. Methylation of Tat by PRMT6 regulates human immunodeficiency virus type 1 gene expression. *J. Virol.* **79**: 124–131.
- Boyer, L.A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L.A., Lee, T.I., Levine, S.S., Wernig, M., Tajonar, A., Ray, M.K., et al. 2006. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441**: 349–353.
- Bracken, A.P., Kleine-Kohlbrecher, D., Dietrich, N., Pasini, D., Gargiulo, G., Beekman, C., Theilgaard-Monch, K., Minucci, S., Porse, B.T., Marine, J.C., et al. 2007. The Polycomb group proteins bind throughout the INK4A–ARF locus and are dissociated in senescent cells. *Genes & Dev.* **21**: 525–530.
- Cook, J.R., Lee, J.H., Yang, Z.H., Krause, C.D., Herth, N., Hoffmann, R., and Pestka, S. 2006. FBXO11/PRMT9, a new protein arginine methyltransferase, symmetrically dimethylates arginine residues. *Biochem. Biophys. Res. Commun.* **342**: 472–481.
- Couture, J.F., Collazo, E., and Trievel, R.C. 2006. Molecular recognition of histone H3 by the WD40 protein WDR5. *Nat. Struct. Mol. Biol.* **13**: 698–703.
- Dou, Y., Milne, T.A., Tackett, A.J., Smith, E.R., Fukuda, A., Wysocka, J., Allis, C.D., Chait, B.T., Hess, J.L., and Roeder, R.G. 2005. Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* **121**: 873–885.
- Dou, Y., Milne, T.A., Ruthenburg, A.J., Lee, S., Lee, J.W., Verdine, G.L., Allis, C.D., and Roeder, R.G. 2006. Regulation of MLL1 H3K4 methyltransferase activity by its core components. *Nat. Struct. Mol. Biol.* **13**: 713–719.
- El-Andaloussi, N., Valovka, T., Toueille, M., Steinacher, R., Focke, F., Gehrig, P., Covic, M., Hassa, P.O., Schar, P., Hubscher, U., et al. 2006. Arginine methylation regulates DNA polymerase β . *Mol. Cell* **22**: 51–62.
- Fabbriozzi, E., El Messaoudi, S., Polanowska, J., Paul, C., Cook, J.R., Lee, J.H., Negre, V., Rousset, M., Pestka, S., Le Cam, A., et al. 2002. Negative regulation of transcription by the type II arginine methyltransferase PRMT5. *EMBO Rep.* **3**: 641–645.
- Frankel, A., Yadav, N., Lee, J., Branscombe, T.L., Clarke, S., and Bedford, M.T. 2002. The novel human protein arginine N-methyltransferase PRMT6 is a nuclear enzyme displaying unique substrate specificity. *J. Biol. Chem.* **277**: 3537–3543.
- Guccione, E., Martinato, F., Finocchiaro, G., Luzi, L., Tizzoni, L., Dall'Olio, V., Zardo, G., Nervi, C., Bernard, L., and Amati, B. 2006. Myc-binding-site recognition in the human genome is determined by chromatin context. *Nat. Cell Biol.* **8**: 764–770.
- Hsieh, J.J., Ernst, P., Erdjument-Bromage, H., Tempst, P., and Korsmeyer, S.J. 2003. Proteolytic cleavage of MLL generates a complex of N- and C-terminal fragments that confers protein stability and subnuclear localization. *Mol. Cell Biol.* **23**: 186–194.
- Hughes, C.M., Rozenblatt-Rosen, O., Milne, T.A., Copeland, T.D., Levine, S.S., Lee, J.C., Hayes, D.N., Shanmugam, K.S., Bhattacharjee, A., Biondi, C.A., et al. 2004. Menin associates with a trithorax family histone methyltransferase complex and with the hoxc8 locus. *Mol. Cell* **13**: 587–597.
- Jenuwein, T. and Allis, C.D. 2001. Translating the histone code. *Science* **293**: 1074–1080.
- Klose, R.J., Yan, Q., Tothova, Z., Yamane, K., Erdjument-Bromage, H., Tempst, P., Gilliland, D.G., Zhang, Y., and Kaelin Jr., W.G. 2007. The retinoblastoma binding protein RBP2 is an H3K4 demethylase. *Cell* **128**: 889–900.
- Lee, J.H. and Skalniak, D.G. 2005. CpG-binding protein (CXXC finger protein 1) is a component of the mammalian Set1 histone H3-Lys4 methyltransferase complex, the analogue of the yeast Set1/COMPASS complex. *J. Biol. Chem.* **280**: 41725–41731.
- Lee, M.G., Wynder, C., Cooch, N., and Shiekhattar, R. 2005. An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* **437**: 432–435.
- Luger, K., Rechsteiner, T.J., Flaus, A.J., Waye, M.M., and Richmond, T.J. 1997. Characterization of nucleosome core particles containing histone proteins made in bacteria. *J. Mol. Biol.* **272**: 301–311.
- Ma, H., Baumann, C.T., Li, H., Strahl, B.D., Rice, R., Jelinek, M.A., Aswad, D.W., Allis, C.D., Hager, G.L., and Stallcup, M.R. 2001. Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. *Curr. Biol.* **11**: 1981–1985.
- Martens, J.H., O'Sullivan, R.J., Braunschweig, U., Opravil, S., Radolf, M., Steinlein, P., and Jenuwein, T. 2005. The profile of repeat-associated histone lysine methylation states in the mouse epigenome. *EMBO J.* **24**: 800–812.
- Milne, T.A., Briggs, S.D., Brock, H.W., Martin, M.E., Gibbs, D., Allis, C.D., and Hess, J.L. 2002. MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol. Cell* **10**: 1107–1117.
- Miranda, T.B., Miranda, M., Frankel, A., and Clarke, S. 2004. PRMT7 is a member of the protein arginine methyltransferase family with a distinct substrate specificity. *J. Biol. Chem.* **279**: 22902–22907.
- Nakamura, T., Mori, T., Tada, S., Krajewski, W., Rozovskaia, T., Wassell, R., Dubois, G., Mazo, A., Croce, C.M., and Canaani, E. 2002. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol. Cell* **10**: 1119–1128.
- Nishioka, K., Chuikov, S., Sarma, K., Erdjument-Bromage, H., Allis, C.D., Tempst, P., and Reinberg, D. 2002. Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. *Genes & Dev.* **16**: 479–489.
- Pal, S., Vishwanath, S.N., Erdjument-Bromage, H., Tempst, P., and Sif, S. 2004. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. *Mol. Cell Biol.* **24**: 9630–9645.
- Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**: e45. <http://nar.oxfordjournals.org/cgi/content/full/29/9/e45>.
- Roh, T.Y., Cuddapah, S., Cui, K., and Zhao, K. 2006. The genomic landscape of histone modifications in human T cells. *Proc. Natl. Acad. Sci.* **103**: 15782–15787.
- Ruthenburg, A.J., Wang, W., Graybosch, D.M., Li, H., Allis, C.D., Patel, D.J., and Verdine, G.L. 2006. Histone H3 recognition and presentation by the WDR5 module of the MLL1 complex. *Nat. Struct. Mol. Biol.* **13**: 704–712.
- Ruthenburg, A.J., Allis, C.D., and Wysocka, J. 2007. Methylation of lysine 4 on histone H3: Intricacy of writing and reading a single epigenetic mark. *Mol. Cell* **25**: 15–30.

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- Schubeler, D., MacAlpine, D.M., Scalzo, D., Wirbelauer, C., Kooperberg, C., van Leeuwen, F., Gottschling, D.E., O'Neill, L.P., Turner, B.M., Delrow, J., et al. 2004. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes & Dev.* **18**: 1263–1271.
- Schurter, B.T., Koh, S.S., Chen, D., Bunick, G.J., Harp, J.M., Hanson, B.L., Henschen-Edman, A., Mackay, D.R., Stallcup, M.R., and Aswad, D.W. 2001. Methylation of histone H3 by coactivator-associated arginine methyltransferase 1. *Biochemistry* **40**: 5747–5756.
- Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstone, J.R., Cole, P.A., Casero, R.A., and Shi, Y. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119**: 941–953.
- Strahl, B.D. and Allis, C.D. 2000. The language of covalent histone modifications. *Nature* **403**: 41–45.
- Takeda, S., Chen, D.Y., Westergard, T.D., Fisher, J.K., Rubens, J.A., Sasagawa, S., Kan, J.T., Korsmeyer, S.J., Cheng, E.H., and Hsieh, J.J. 2006. Proteolysis of MLL family proteins is essential for taspase1-orchestrated cell cycle progression. *Genes & Dev.* **20**: 2397–2409.
- Tang, J., Gary, J.D., Clarke, S., and Herschman, H.R. 1998. PRMT 3, a type I protein arginine N-methyltransferase that differs from PRMT1 in its oligomerization, subcellular localization, substrate specificity, and regulation. *J. Biol. Chem.* **273**: 16935–16945.
- Torres-Padilla, M.E., Parfitt, D.E., Kouzarides, T., and Zernicka-Goetz, M. 2007. Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature* **445**: 214–218.
- Turner, B.M. 2002. Cellular memory and the histone code. *Cell* **111**: 285–291.
- Wagner, S., Weber, S., Kleinschmidt, M.A., Nagata, K., and Bauer, U.M. 2006. SET-mediated promoter hypoacetylation is a prerequisite for coactivation of the estrogen-responsive pS2 gene by PRMT1. *J. Biol. Chem.* **281**: 27242–27250.
- Wang, H., Cao, R., Xia, L., Erdjument-Bromage, H., Borchers, C., Tempst, P., and Zhang, Y. 2001a. Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Mol. Cell* **8**: 1207–1217.
- Wang, H., Huang, Z.Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B.D., Briggs, S.D., Allis, C.D., Wong, J., Tempst, P., et al. 2001b. Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* **293**: 853–857.
- Wysocka, J., Myers, M.P., Laherty, C.D., Eisenman, R.N., and Herr, W. 2003. Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1. *Genes & Dev.* **17**: 896–911.
- Wysocka, J., Swigut, T., Milne, T.A., Dou, Y., Zhang, X., Burlingame, A.L., Roeder, R.G., Brivanlou, A.H., and Allis, C.D. 2005. WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* **121**: 859–872.
- Wysocka, J., Swigut, T., Xiao, H., Milne, T.A., Kwon, S.Y., Landry, J., Kauer, M., Tackett, A.J., Chait, B.T., Badenhurst, P., et al. 2006. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* **442**: 86–90.
- Xiao, B., Jing, C., Wilson, J.R., Walker, P.A., Vasisht, N., Kelly, G., Howell, S., Taylor, I.A., Blackburn, G.M., and Gamblin, S.J. 2003. Structure and catalytic mechanism of the human histone methyltransferase SET7/9. *Nature* **421**: 652–656.

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Dawin Hyllus, Claudia Stein, Kristin Schnabel, et al.

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