Cross-talk between Histone Modifications in Response to Histone Deacetylase Inhibitors

**MLL4 LINKS HISTONE H3 ACETYULATION AND HISTONE H3K4 METHYLATION**

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Histones are subject to a wide variety of post-translational modifications that play a central role in gene activation and silencing. We have used histone modification-specific antibodies to demonstrate that two histone modifications involved in gene activation, histone H3 acetylation and H3 lysine 4 methylation, are functionally linked. This interaction, in which the extent of histone H3 acetylation determines both the abundance and the "degree" of H3K4 methylation, plays a major role in the epigenetic response to histone deacetylase inhibitors. A combination of *in vivo* knockdown experiments and *in vitro* methyltransferase assays shows that the abundance of H3K4 methylation is regulated by the activities of two opposing enzyme activities, the methyltransferase MLL4, which is stimulated by acetylated substrates, and a novel and as yet unidentified H3K4me3 demethylase.

This suggests that epigenetic marks are not deposited or recognized in isolation but comprise a complex and inter-related collection of modifications at adjacent residues. The correlation between different histone modifications is particularly clear for histone H3 acetylation and the methylation of histone H3 lysine 4 (H3K4me). Mass spectrometric analysis of the cellular pool of histones indicates that this methyl mark is associated with histone H3 molecules containing high levels of acetylation (9). This is consistent with the observed co-localization of these marks, which show related distribution patterns both at a chromosome-wide level during X inactivation (10) and over the coding regions of individual genes (11, 12). These correlations may arise due to physical links between histone-modifying enzymes such that they are co-recruited to the same loci. Both MLL1, a histone methyltransferase (HMT) that can generate H3K4me marks (13), and Chd1, the chromatin remodeler that is subsequently recruited by this methyl mark, associate with histone acetyltransferase activities (14, 15), whereas the LSD1 complex that removes some of these methyl marks contains the histone deacetylases HDAC1 and HDAC2 (16). However, the interaction could also arise due to the mechanism of action of these enzymes. For example, the SET domain of MLL1 has a preference for acetylated substrates (13).

A growing body of evidence suggests that many different types of post-translational histone modifications play key roles in regulating gene expression and that some modifications at least are functionally inter-related (1). The linked deposition of distinct modifications can occur both on the same histone tail, e.g. H3S10 phosphorylation and H3K9 acetylation (2) or on different tails, e.g. H2A ubiquitination and H3 methylation (3), histone acetylation, and methylation (4, 5). Multiprotein complexes have been identified that are capable of depositing, or removing, different modifications in a coordinated manner (e.g. histone demethylase and deacetylase activity in coREST) (6). Similarly, binding proteins are sensitive to combinations of modifications; for example, HP1 binding to the H3 tail requires histone H3K9 methylation but is blocked by H3S10 phosphorylation and H3K14 acetylation (Ref. 7, although see Ref. 8).

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† The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

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§ The abbreviations used are: HMT, histone methyltransferase; HDAC, histone deacetylase; HDI, histone deacetylase inhibitor; PBS, phosphate-buffered saline; siRNA, small interfering RNA; TAU, Triton-acid-urea.

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and a novel, and as yet unidentified, H3K4me3 demethylase activity.

**EXPERIMENTAL PROCEDURES**

**Tissue Culture and Reagents**—HeLa and HL60 cells were grown in RPMI medium supplemented with 8% fetal bovine serum (Invitrogen), L-glutamine (Invitrogen), and penicillin (Invitrogen). Histone hyperacetylation was induced by exposure of cells to sodium butyrate (10 mM final concentration), sodium valproate (5 mM), or Trichostatin A (165 nM) for 6 h.

**Histone Extraction, Gel Systems, and Western Blotting**—Histones were extracted from tissue culture cells (1–2 × 10^7) by acid extraction. Briefly, cells were harvested by centrifugation, washed twice with ice-cold PBS containing 5 mM sodium butyrate, and lysed in Triton extraction buffer, TEB (PBS + 0.5% Triton X-100). After centrifugation, the supernatant was discarded and the nuclear pellet was washed again with TEB prior to the histones being extracted with 0.2N HCl on ice overnight. Histones were separated on 15% acrylamide/bis-acrylamide gel filtration column (GE Healthcare). GST-MLL1 SET fusion protein was expressed in E. coli BL21(DE3) by induction with 0.1 mM isopropyl-β-D-thiogalactopyranoside at 22 °C. The enzyme was purified over a glutathione-Sepharose 4B column, followed by a Tricorn Superdex 200 10/30 GL gel filtration column (GE Healthcare). GST-MLL1 SET was eluted with buffer containing 50 mM Tris-Cl, pH 8.5, and 100 mM NaCl and stored at −80 °C after the addition of dithiothreitol and glycerol to a final concentration of 1 mM and 10%, respectively.

**Methylation Assays**—Biotinylated peptides corresponding to the first 21 amino acids of the human histone H3 N-terminal tail (50 µl of a solution of 5 µg/µl in PBS) were immobilized onto streptavidin-coated 96-well microtiter plates (Sigma) during 1 h at room temperature. After washing with water (three times), the wells were incubated with 200 µl of 3% bovine serum albumin in PBS for 2 h at room temperature. Subsequently, the wells were incubated with PBS (three times) and incubated with 50 µl of methylation reaction mixture (1 µM methyltransferase, 0.5 mM S-adenosyl methionine (Fluka; purity ≥80%, stored in 10 mM H_2SO_4 at −20 °C), 50 mM Tris-Cl, pH 8.5, 100 mM NaCl, 2 mM dithiothreitol) or the control reaction (0.5 mM S-adenosyl methionine, 50 mM Tris-Cl, pH 8.5, 100 mM NaCl, 2 mM dithiothreitol). The plates were incubated for the indicated time intervals at 30 °C and washed with PBS (three times), and the enzymatically modified peptides were incubated with 50 µl of anti-H3K4Me1, anti-H3K4Me2, or anti-H3K4Me3 antibody (diluted in 3% bovine serum albumin, PBS) for 1 h at room temperature. The wells were then washed with PBST (PBS-0.5% Tween-20)/500 mM NaCl (two times), PBST (two times), PBS (three times) and incubated with an anti-rabbit IgG horseradish peroxidase conjugate (Sigma) for 1 h at room temperature, followed by washing with PBST (three times) and with water (three times). After incubation with 100 µl of horseradish peroxidase substrate (TMB Microwell Peroxidase Substrate; KPL) for 10 min, 50 µl of 1 M H_2SO_4 were added and the absorbance at 450 nm measured on a SpectraMaxPlus reader (Molecular Devices). For each peptide substrate, the values obtained without enzyme were defined as background methylation and subtracted from those obtained for the enzymatic methylation.

**Peptides**—Synthetic peptides used in the methylation assays correspond to amino acids 1–21 of human histone H3 followed by a GG linker and biotinylated lysine (Upstate).
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and the methylation of specific lysines. Logarithmically growing HL60 cultures incubated with 6 mM sodium valproate (Fig. 1, A and B) or other HDAC inhibitors (2.5 μM SAHA, 10 mM butyrate; data not shown) show, as expected, a rapid and sustained increase in histone acetylation. The magnitude of the response varies with the acetyl isoform examined, from an ~8-fold increase in H4K8ac to an ~24-fold increase in H3K9/K18ac (Fig. 1, A and B), reflecting the abundance of these histone acetyl isoforms in untreated cells and the differing rates of acetyl turnover at different lysine residues (21). HDAC inhibitors also induce a concomitant increase in methylation at H3K4, the degree of enhancement depending on the specific methyl isoform; H3K4me3 increases ~5-fold and H3K4me2 levels increase ~3-fold, whereas H3K4me1 levels show only a small increase (1.5 to 2-fold, Fig. 1B). In contrast, none of the inhibitors enhanced levels of mono-, di-, or tri-methylation at H3K9 or H4K20, modifications associated with transcriptional repression (data not shown). HDAC inhibitors have induced the same specific increase in H3K4 methylation in all cell types tested so far, including HL60 cells, HeLa cells, mouse embryonic stem cells (data not shown), and primary blast cells from patients with acute myeloid leukemia (22).

Histone H3 Acetylation Directly Impacts on the Degree of Methylation of H3K4—Experiments treating tissue culture cells with HDAC inhibitors show a correlation between the extent of histone acetylation in a cell and the abundance of histone tri- and di-methylation at H3K4. We next addressed whether the increased acetylation and H3K4 methylation induced by HDAC inhibitors occur on the same H3 molecules by examining histones on acid-urea gels that resolve the distinct histone H3 acetyl isoforms (Fig. 2). Untreated cells contain a mixture of non-acetylated, mono- and di-acetylated H4, whereas treated cells contain highly acetylated histone isoforms up to tetra-acetylated H4 (Fig. 2, right panel). Histones separated on acid-urea gels were then Western blotted and probed with antibodies specific to distinct H3K4 methyl isoforms (H3K4me1, 2, and 3).

A central observation from this experiment is that in untreated cells the bulk of H3K4 methylation (labeled H3K4me1, 2, or 3 in Fig. 2) is associated with acetylated histones rather than non-acetylated histone H3, the isoform that predominates in these cells (Fig. 2, Silver stain). This suggests that at least one acetyl group is required on H3 for Lys-4 methylation to occur. A second observation is that in treated cells the different degrees of Lys-4 methylation are associated with different levels of H3 acetylation; H3K4me1 is primarily found on di- and tri-acetylated H3, H3K4me2 on di-, tri-, and tetra-acetylated H3 and H3K4me3 on tri-, tetra-, and penta-acetylated H3. These observations are consistent with the distribution of marks revealed by mass spectrometry (9) and suggest not only that H3 acetylation is required for H3K4 methylation to occur but that the degree of methylation induced by HDIs is determined by the acetylation level of distinct H3 molecules. This has a number of implications, which are discussed later.

HDAC Inhibitors Induce Changes in the H3 Isoform Distribution—Histone H3 exists as three closely related isoforms in eukaryotic cells (H3.1, H3.2, and H3.3), distinguished by just a few highly conserved substitutions in the primary amino acid sequence. Recent studies indicate that H3.3 is associated with a distinct chromatin assembly complex (HIRA) (23), is enriched in regions of actively transcribed chromatin, and is associated with “activating” post-transcriptional modifications, including H3K4me3 (24). It

![FIGURE 1. HDAC inhibitors stimulate substantial increases in both histone H3 acetylation and Lys-4 methylation. Western blots (A) and quantitative presentation (B) of the changes in distinct histone modifications in HL60 cells over a 24-h incubation with sodium valproate (6 mM). Acetylation of both histones H4 (H4K8ac) and H3 (H3K9/18ac) and the three distinct H3K4 methyl marks H3K4me1, H3K4me2, and H3K4me3 are presented.](image)

![FIGURE 2. The abundance and degree of methylation at histone H3K4 is linked to H3 acetylation status. Acid-urea (AU) gels were used to separate the histones extracted from control (−) and butyrate-treated (+) HL60 cells and then either silver stained to show the changed distribution of acetyl-histone isoforms or probed with methyl-specific antibodies (H3K4me1/2/3). Distinct histone H4 or H3 acetyl isoforms are indicated, with unmodified H4 (0), mono- (1), di- (2), tri- (3), and tetra-acetylated histone H4 on the silver-stained gel (right panel) or acetyl-H3 isoforms indicated in the Western blots (un-modified H3 (0); mono-acetyl H3 (1), etc.).](image)
was therefore essential to examine whether the changes induced by HDAC inhibitors were associated with changes in either the abundance or modification levels of the distinct H3 isoforms. Resolution of histones from control and HDI-treated HL60 cells on two-dimensional TAU-SDS gels indicates that, whereas H3.1 and H3.2 represent the most abundant H3 isoforms, inhibitor treatment increases the proportion of H3.3 in chromatin, suggesting that either histone acetylation or the subsequent functional changes that occur in the nucleus encourage its deposition (Fig. 3A, arrows). This is a significant change, albeit from a very minor fraction of the H3 in untreated cells, as the bulk of the chromatin remains associated with H3.1 and H3.2 (Fig. 3A, + Butyrate).

Two-dimensional gels resolving histones from control and inhibitor-treated cells were Western blotted and probed with antibodies against mono- and tri-methyl H3K4 to assess the distribution of these marks on the distinct histone H3 isoforms (Fig. 3B). This shows that in untreated cells the bulk of both H3K4me1 and H3K4me3 are evenly distributed between H3.1 and H3.2, with little methylation associated with H3.3 (Fig. 3B, left panels). This is surprising, as it does not reflect the relative proportions of these isoforms in the histone pool where H3.1 is clearly predominant (Fig. 3A), and indicates that H3.2 is relatively enriched in these methyl marks. The H3 Lys-4 methyl marks are also unequally distributed after inhibitor treatment, with the observed increase in H2K4me3 largely restricted to H3.1 and H3.2. In contrast, the newly deposited H3.3 is largely marked with H3K4me1 (Fig. 3B). This shows that the H3K4me2/3 marks induced by HDAC inhibitors are unlikely to reflect the de novo assembly of H3.3 into chromatin.

Histone H3K4 Methyl Isoforms Are Subject to Rapid Demethylation—Histone methylation has been historically thought of as a long-lasting modification and until recently was thought to be removed largely by replacement of methylated with unmethylated histone (i.e. H3.3) (25)). Given our current results that indicate that histone H3K4 methylation is linked to the extent of histone acetylation, we wanted to examine the stability of this mark when histone acetylation levels fall. To generate these conditions, tissue culture cells were exposed to HDAC inhibitors to induce high levels of histone acetylation and H3K4me3/2; the inhibitor was subsequently removed, and the resultant changes in histone modifications were examined by Western analysis. Removal of the HDAC inhibitor induced a rapid and substantial decrease in histone acetylation (H4K8ac), consistent with the rapid turnover of this mark in chromatin (H3K4me3/2; Fig. 4A). Global histone deacetylation was accompanied by the removal of HDI-induced H3K4 methylation, with the extent of demethylation of specific methyl marks mirroring their original HDI-mediated stimulation (H3K4me3 >me2 >me1; Fig. 1A). Interestingly, demethylation is also rapid, with the removal of H3K4me3 and H3K4me2 at similar rates (H3K4me3 t_{1/2} 53 min; H3K4me2 t_{1/2} 57 min., indicating these methyl marks are subject to moderate rates of turnover.

Acid-urea gel analysis of the histones post-washout indicates H3 deacetylation and K4me3 demethylation act in parallel on the same histone molecules (Fig. 4B). However, a significant proportion of the H3K4me3 is associated with mono- and non-acetylated histone H3 at intermediate time points. This is significant as it indicates that the processes of deacetylation and demethylation are occurring on the same histone molecules; replacement of methylated histones with non-methylated H3.3
would not generate these “intermediate” acetyl-methylated H3 isoforms. The observation that a significant proportion of tri-methyl marks are observed on non-acetylated histone under these conditions is also notable as this isom is not seen in untreated cells (Fig. 2). This suggests that the process of deacetylation acts faster than K4me3 demethylation on individual histone molecules, consistent with the changes observed in the global histone pool (Fig. 4.A).

MLL4 Is the Primary Mediator of H3 Acetylation-H3K4me3

Cross-talk—The data presented indicate that HDIs induce a substantial increase in H3K4 methylation and that this increase is directly linked to the acetylation status of individual H3 molecules. However, the molecular basis of this “cross-talk” is unclear, particularly given the complexity of the observed marks. H3K4 is a substrate for several methyltransferases, and the mechanism of their activity, beyond whether they generate mono-, di-, or tri-methylated products, is largely unexplored. However, given the observation that the activity of the SET domain of MLL1, a H3K4 methyltransferase, is stimulated by acetylated substrates (13) we examined whether this activity or that of related enzymes was the basis of the observed cross-talk in response to HDIs.

Initial experiments using reverse transcription PCR against unique regions of a range of H3K4-specific methyltransferases (supplemental Table S1) confirmed that HeLa cells contain broadly similar mRNA levels of individual members of the MLL family of methyltransferases (MLLs 1–5) and SET-7, a distinct H3K4 HMT that can only generate mono-methylated marks (19) (data not shown). We were unable to address the corresponding protein abundance as specific antibodies are unavailable. We then used a siRNA knockdown approach to identify the histone methyltransferase(s) responsible for the HDI-induced increase in H3K4 methylation. Plasmids expressing siRNAs for MLL 1–5 and SET-7 were transfected into HeLa cells. Cells were grown for 48 h to ensure efficient knock down and subsequently incubated with sodium butyrate. The inhibitor induced a substantial and equivalent increase in histone acetylation in both control (mock transfected) and all the “knockdown” cells as assessed by AUT gels (data not shown). As expected, there was a significant increase in H3K4 methylation in the mock-transfected controls following butyrate treatment. Knock down of SET-7 and MLL1, 2, 3, and 5 did not prevent this butyrate-induced increase of H3K4me2/3 (Fig. 5, A and B), suggesting that these methyltransferases do not contribute to the global increase in H3 methylation. In contrast, knock down of MLL4 essentially abrogated the increase in H3K4me3/2 (Fig. 5, A and B). These knock downs have each been repeated three times with consistent results.

In Vitro Methylation Assays Confirm That the MLL SET Domain Responds to Histone Modifications—The finding that MLL4 is the major activity responsible for the preferential addition of K4me3 to acetylated histone H3 raised a number of questions regarding the mechanism by which the distinct isoforms are generated in chromatin. The homologous HMT, MLL1, is known to generate tri-methylated marks (26), and the HMTase activity of its SET domain is known to be stimulated by acetylated substrates (13). Significantly, the SET domains of MLL1 and MLL4, which are the domains responsible for the methyltransferase activity of the proteins, are highly related (>85% sequence similarity, 75% sequence identity). As we were unable to express the SET domain of MLL4, we utilized in vitro methylation assays to assess whether an Escherichia coli-expressed SET domain from MLL-1 could reconstitute the distribution of H3 methyl isomers observed in vivo.

Initial experiments examined the HMTase activity of the SET domain from MLL1 and the activity of the mono-methyl HMT SET-7 on non-acetylated and di-acetylated (K9/K14ac) H3 peptide substrates. The levels of specific methyl isoforms generated were assayed with methyl-specific antibodies. Both enzymes showed a linear accumulation of methylated substrate over the experiment time course (Fig. 6, A and B), but the rate of MLL SET domain HMTase activity and the final absolute level of all three methyl marks (H3K4me1, 2, and 3) are substantially increased on acetylated substrates (Fig. 6A). Interestingly, the data show that the rates of generating the individual methyl marks are different, with higher levels of H3K4me1 deposition and discernable “lag-phases” in the generation of the di- and tri-methyl marks (Fig. 6A), suggesting that MLL-induced methylation acts processively. In contrast, SET-7 mono-methyl transferase activity is not stimulated on the acetylated template (Fig. 6B), showing that not all SET domains are influenced by the acetylation status of their substrate.

The observation that MLL SET domain HMTase activity is stimulated by the di-acetylation of the substrate prompted us to examine the effects of a number of modifications present on the H3 tail. Methylation assays were performed with a range of mod-
ified H3 substrates, namely mono-acetylated (H3K9ac and H3K14ac), di-acetylated (H3K9acK14ac), phosphorylated (H3S10ph), and phospho-acetylated (H3K9acS10phK14ac). These data are consistent with our observation that H3 acetylation stimulates SET domain HMTase activity, with similar increases in methyl deposition seen on mono- and di-acetylated templates (Fig. 6C). Interestingly, H3 serine 10 phosphorylation also stimulates methylase activity to a similar extent. However, the most striking result is that phospho-acetylation (H3K9acS10phK14ac) of the substrate results in a substantial increase (2 to 3-fold) in the deposition of all methyl marks. A final experiment examining SET-7 MTase activity on the same series of multiply modified peptides did not show significant differences in the methylation of different substrates (i.e. within experimental error), reinforcing our earlier findings (Fig. 6D). These data also confirm the specificity of the anti-H3K4me antibodies used; binding is clearly not affected by the presence of adjacent acetyl or phosphoryl residues.

In summary, both histone H3 acetylation and H3S10 phosphorylation facilitate the deposition of each distinct methyl mark to different extents (i.e. stimulating K4me1 >me2 >me3), suggesting that the initial methylation step (H3K4 to H3K4me1) is the most sensitive to substrate modification.

**DISCUSSION**

**A Link between H3 Acetylation and H3K4 Methylation**—The functional role(s) of histone H3 acetylation and H3K4 methylation broadly correlate. Enhanced levels of both H3 acetylation and H3K4me3 are detected at the transcriptional start sites of active promoters (12), and both marks exert functional effect(s) by recruiting activating chromatin enzymes through interactions with bromo- and chromo-domain-containing proteins (14, 15). This correlation is also observed biochemically in the global pool of histones; mass spectrometry (9) and Western blot analysis (22, 27) demonstrate that the level of histone H3 acetylation is linked to the degree of methylation at H3 lysine 4.

The experiments presented here show that conditions that increase histone H3 acetylation (i.e. treatment with deacetylase...
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inhibitors) are associated with increased levels of H3K4 methylation, primarily the di- and tri-methylated forms, whereas conditions that decrease H3 acetylation (i.e. removal of deacetylase inhibitors) also reduce the abundance of these methyl marks. For bulk histones and over a time course of 1–6 h after the addition/removal of HDIs, the level of H3 acetylation is tightly linked to the level of H3K4 methylation. This interaction is not restricted to cells treated with HDAC inhibitors, as in untreated cells H3K4 methylation is seen almost exclusively on acetylated (primarily mono-acetylated) H3 isoforms and was virtually absent from the more common non-acetylated form. Further, we note that the degree of methylation is linked to the extent of acetylation, with H3 isoforms mono-, di-, and tri-methylated at Lys-4 being associated with progressively more highly acetylated H3 isoforms (Fig. 2) (9).

Two related observations suggest that the level of histone H3 acetylation is causal for or determines the degree of H3K4 methylation. 1) Kinetic data indicate that changes in histone acetylation precede changes in histone methylation. This is clear from both the initial rates of H3K9/18ac and H4K8ac increase and H4K8ac decrease compared with all H3K4me marks following stimulus (addition or removal of HDIs) (Figs. 1 and 4). 2) Similarly, time course analysis of the changes in acetyl-methyl H3 isoforms following washout on TAU gels demonstrates that histone H3 deacetylation proceeds faster than demethylation. HDI washout results in histones with unusual patterns of modifications, i.e. H3K4 me3 marks associated with histones with low levels of acetylation. These isoforms are not seen in untreated or HDI-treated cells, but their occurrence is easily explained by the finding that acetyl groups are more rapidly removed from histone H3 than methyl groups following removal of the HDI (Fig. 4A). It is unlikely that they result from de novo deposition of H3.3, given that this histone isoform is primarily mono-methylated at H3K4 in chromatin (Fig. 3).

This conclusion is in contrast with another study of histone modification changes associated with the rapid induction of immediate early genes. Hazzalin et al. (32) also noted a link between acetylation and H3K4 methylation but presented evidence to suggest that acetylation was selectively targeted to chromatin in which H3K4 was already tri-methylated. Such selective targeting of acetylation may account for changes at the level of individual genes but does not explain the global increase in H3K4 methylation noted here in response to HDAC inhibitors or its loss following their withdrawal.

Mechanistic Basis of Acetylation: Methylation Cross-talk—
The observation that H3 acetylation and H3K4 methylation are linked gives an insight into the enzymology underlying the deposition and removal of these modifications. Cellular levels of histone acetylation reflect the balance of histone acetyltransferase and HDAC activities recruited at numerous loci throughout the genome. In contrast, the enzymology of histone lysine methylation is less clear, partially because of the increased complexity of methyl-lysine marks in chromatin. Mono, di-, and tri-methyl lysine act as distinct epigenetic marks and may be generated by a single enzyme acting processively or by the sequential action of several methyltransferases. The recent identification of lysine-specific demethylases (LSD1 and JHDM1, (6, 27–31) indicates that enzymatic removal of these marks can occur, yet a tri-methyl demethylase specific for H3 lysine 4 remains to be identified.

The data presented here represent the first indication that an enzymatic H3K4 tri-methyl demethylase activity exists (possibly in the context of a histone demethylase with wider substrate specificity) and that histone replacement is not required to remove this mark from chromatin. H3K4me3 marks are rapidly removed from chromatin upon removal of HDIs (Fig. 4), correlating with the rapid loss of global histone H3 acetylation (Fig. 4, A and B). These data, and the rapid removal of an abundant mark (i.e. ~90% of penta-acetylated H3 is tri-methylated at H3K4) (9), are consistent with an enzymatic H3K4me3 demethylase rather than H3.3 replacement. As such, this suggests that the regulation of histone methylation is broadly similar to that of histone acetylation, governed by opposing classes of enzymes that are likely to be recruited to target loci by protein–protein interactions.

Using siRNA knockdown technology we demonstrate that the methyltransferase MLL4, but not the related enzymes MLL1, 2, 3, or 5 (Fig. 5), is responsible for the interaction between acetylation and H3K4 methylation that we observe in global chromatin. The specificity for MLL4 is perhaps surprising given that MLL1 and MLL4 are closely related, but it may reflect functional differences in the complexes they form or their abundance or spatial distribution.

Cross-talk and Enzyme Complexes—Histone-modifying enzymes are typically found in multisubunit complexes with other enzymatic activities, and this may be the basis of many forms of epigenetic cross-talk. This appears to be the case for lysine methylation; MLL1 (HRX-1/ALL-1) is a component of a large multisubunit complex containing both the histone acetyltransferase activities MOF (26) and CBP (27) and WDR5, a WD40-repeat protein that targets the complex to H3K4me sites (28). Similarly, coREST, the complex containing the H3K4 demethylase LSD1, contains both HDACs and 2 (6, 29). This suggests HDIs may also target HDAC1 and 2 in this complex. In preliminary experiments we examined the contribution of the coREST complex to cellular levels of acetylation and H3K4 methylation by using the mono-amine oxidase inhibitor translycypromine (“Parnate”) (30). We found 6 h of exposure to 100 μM Parnate induced small but significant increases in H3K4 methylation (1.2-fold), consistent with previous studies (30). Interestingly, we also saw a modest increase in H3 acetylation (H3K9/18ac, 1.7-fold), suggesting that HDAC1 and 2 in the context of the coREST complex do contribute to the cellular levels of histone acetylation. However, the magnitude of these changes suggests that the coREST complex plays only a small part in regulating the global pool of histone acetylation and H3K4 methylation.

These observations suggest that both the deposition or removal of H3K4 methyl marks by enzyme complexes is coincident with the deposition or removal of acetyl marks at adjacent sites. The observation that a large proportion of both tetra- and penta-acetylated histone H3 is methylated at H3K4 (~50 and ~90%, respectively) (9) is consistent with this and suggests that cross-talk between these marks is the norm, albeit at those restricted loci containing highly acetylated histones. This gen-
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The abundance of specific methyl isoforms at H3 lysine 4 (me1, me2, or me3) is linked to the level of H3 acetylation. We propose that this is under the regulation of several opposing multienzyme complexes; a MLL4-histone acetyltransferase complex is responsible for depositing these marks, and a novel H3 K4me3 demethylase-HDAC complex, either alone or in combination with the LSD1 coreST complex, is responsible for removing them. Importantly, the H3 tail must be acetylated for subsequent methyltransferase activity.

The delayed availability of deacetylated substrate may explain the slower rate of demethylation observed (t1/2 53 min). It is interesting to note that demethylation of tri- and di-methyl marks proceeds at essentially the same rate, suggesting that this is a single processive mechanism.

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