folding. It is also possible that the more drastic mutations of Chodaparambil *et al.*[5] lead to changes in the nucleosome surface beyond the charged pocket. It would be interesting for future experiments to further explore potential differential effects of specific residues within the charged pocket and whether other regions on the protein surface of the nucleosome have distinct roles in chromatin condensation. At any rate, these studies point to a complicated set of interactions involving the H4 tail domain that affect the stability of both secondary and tertiary chromatin structures.

In summary, these two studies highlight a heretofore underappreciated mechanism for regulating chromatin structure. Although the binding of LANA may not have a specific effect on transcription, one can easily imagine how a comparable region within a targeted transcription factor or regulatory protein may use a similar mechanism to elicit a specific functional state in a region of chromatin. Moreover, an analogous mechanism seems to distinguish chromatin containing H2A.Bbd and H2A.Z. It will be interesting to determine whether other variants of H2A or H2B[15] affect the charged pocket and chromatin condensation and whether as yet unidentified post-translational modifications occur within the charged pocket to tune such interactions. Don't be surprised to find newly identified pocket-binding proteins making appearances in your favorite journal in the near future.

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Histone H3 Arg2 methylation provides alternative directions for COMPASS

Robert J Klose & Yi Zhang

Post-translational modification of histones can profoundly affect chromatin structure and function. The discovery that histone H3 Arg2 methylation is a widespread silencing modification that inhibits histone H3 Lys4 trimethylation extends our understanding of how active and silenced chromatin states are maintained.

The nucleosome forms the basic building block of chromatin and consists of 146 base pairs of DNA wrapped around a histone octamer[1]. In addition to compacting DNA within the nucleus, histones also serve as a canvas for post-translational modifications that regulate and dictate chromatin function. In particular, the N-terminal tails of histone molecules protrude from the nucleosome and can be modified by phosphorylation, methylation, ubiquitination and acetylation[2]. Two recent studies published in *Nature*[3,4] report the discovery that asymmetric dimethylation of H3 Arg2 (H3R2me2a) is generally distributed throughout regions of silenced chromatin. This modification counteracts the active transcriptional state by blocking the function of the H3 Lys4 (H3K4) methyltransferase complexes COMPASS in yeast and ASH2/MLL in humans.

Histone methylation is one of the most pervasive histone modifications, and occurs on both arginine and lysine residues[5]. Histone arginine methylation is generally associated with transcriptionally active chromatin, whereas histone lysine methylation is found in both silenced and active regions of chromatin[6]. Unlike many other histone modifications, methylation does not alter the overall charge of the lysine or arginine residues and therefore is not thought to directly alter interactions between histone molecules and DNA. Instead, recent work has demonstrated that histone methylation is recognized by a growing list of effector proteins[7]. Histone arginine residues can be monomethylated (me1), asymmetrically dimethylated (me2a) or symmetrically dimethylated (me2s), and histone lysine residues can be mono- (me1), di- (me2) or trimethylated (me3). The fact that effector proteins recognize defined methylation states effectively increases the complexity of information that can be stored in each individual methylated residue. In addition, effector protein recognition can be regulated by the combination of individual modifications on a histone molecule[8]. These sophisticated recognition properties of effector proteins correspond to the complexity of the histone methylation signal and demonstrate the extensive functional interplay between post-translational histone modifications[8].

It has recently been demonstrated that H3R2me2a is a feature of human genes[9] and is also present in mice during their early development[10]. To further characterize the role of H3R2 methylation, Kirmizis *et al.*[3] have used genome-wide location analysis (GWLA) in budding yeast. They demonstrate that H3R2me2a is found throughout both heterochromatic and euchromatic regions (**Fig. 1a**). To understand the role of H3R2me2a in budding yeast, the authors

Robert J. Klose is in the Department of Biochemistry at the University of Oxford, Oxford, OX1 3QU, UK, and Yi Zhang is in the Howard Hughes Medical Institute, Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Centre, University of North Carolina, Chapel Hill, North Carolina, 27599-7295, USA.
e-mail: rob.klose@bioch.ox.ac.uk or yi_zhang@med.unc.edu

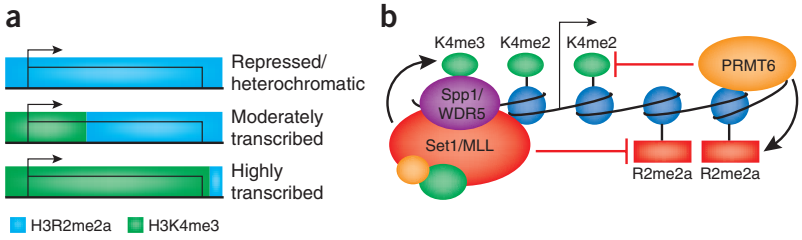

Figure 1 H3R2me2a is found in silenced regions of chromatin and inhibits H3K4me3. (**a**) Genome-wide location analysis in budding yeast reveals that H3R2me2a-modified chromatin is enriched in heterochromatic regions and in the bodies of moderately transcribed genes. Chromatin containing H3R2me2a is mutually exclusive with chromatin containing H3K4me3, suggesting a negative functional interplay between these modifications. (**b**) The budding yeast COMPASS and human MLL/ASH2 H3K4 methyltransferase complexes cannot methylate histone H3 containing H3R2me2a. The PRMT6 H3R2me2a methyltransferase cannot methylate chromatin that contains H3K4me3. Distinct chromatin-recognition properties of H3R2 and H3K4 methyltransferases may help to delineate and maintain active and silent chromatin states.

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generated mutant yeast strains in which H3R2 was substituted with residues that are refractory to methylation (H3R2A/Q). These mutant strains had defects in normal heterochromatin silencing, indicating that H3R2me2a may have a role in maintaining transcriptionally silent chromatin. In telomeric chromatin, loss of H3R2me2a did not result in displacement of the repressive telomeric proteins Rap1 and Sir2, indicating that H3R2me2a contributes to silencing through a previously unrecognized mechanism. In euchromatic regions, the presence of H3R2me2a was inversely correlated with transcriptional activity and skewed toward the 3′ ends of actively transcribed genes (**Fig. 1a**). Consistent with a role for H3R2me2a in transcriptional silencing, inducible gene expression occurred more rapidly in the mutant H3R2A strain, suggesting that H3R2me2a has effects that constrain temporal gene expression.

In budding yeast, a particular lysine methylation mark, H3K4me3, is found at actively transcribed genes and is generally a feature of the 5′ end of the gene. Comparison of the distributions of H3R2me2a and H3K4me3 revealed a very striking inverse correlation (**Fig. 1a**). In transcribed genes, H3R2me2a was lost from the 5′ end of the gene, and H3K4me3 and H3R2me2a were mutually exclusive, suggesting a potential negative interplay between these modifications. Interestingly, purified COMPASS H3K4 methyltransferase complex was unable to methylate H3K4 in substrates containing H3R2me2a, and the H3R2A mutant yeast strain showed a global loss of H3K4me3 but not of H3K4me1 or H3K4me2. This suggests that the presence of an unmodified H3R2 residue is important for transition from the H3K4me2-modified state to the H3K4me3-modified state. The Spp1 component of the COMPASS complex is required for efficient H3K4me3 methyltransferase activity[11]. Spp1 may contribute to enzymatic activity through its capacity to recognize H3K4me2-modified histones using a PHD domain[12]. Interestingly, *in vitro* binding assays with the recombinant Spp1 PHD domain have demonstrated that H3R2me2a disrupts recognition of H3K4me2 and H3K4me3 by Spp1 (ref. 3). This observation provides a simple molecular explanation for the loss of COMPASS activity toward H3R2me2a substrates *in vitro*. Together with the loss of the H3K4me3 modification in H3R2A mutant yeast strains, these results represent compelling evidence that trimethylation of H3K4 is directly inhibited by H3R2me2a (**Fig. 1b**).

In a related study, Guccione *et al.*[4] used a quantitative chromatin immunoprecipitation (ChIP) approach to detect H3R2me2a and H3K4me3 on a subset of promoters in two different human cell lines. In agreement with their previous studies[9], the authors found a negative correlation between these two modifications and a negative correlation between H3R2me2a promoter methylation and transcription. Previous work has demonstrated that mouse embryos deficient in Prmt4 (also called Carm1) lack H3R2me2a during early development and that recombinant PRMT4 can methylate H3R2 *in vitro*[10]. This suggests that the PRMT4 methyltransferase enzyme is responsible for catalyzing the asymmetric dimethylation of H3R2. To examine this possibility, Guccione *et al.*[4] used short interfering RNA (siRNA) to knock down six of the human protein arginine methyltransferases (PRMT1–PRMT6). Surprisingly, PRMT4 knockdown did not affect H3R2me2a levels; instead, PRMT6 knockdown had a large effect on the global levels of H3R2me2a. Furthermore, *in vitro* experiments with recombinant PRMT6 also revealed that this methyltransferase enzyme was capable of producing H3R2me2a. Therefore, in the cell lines used for this study, PRMT6 is responsible for the majority of H3R2me2a.

Given the negative correlation between H3K4me3 and H3R2me2a at human promoters, the authors examined the capacity of the human ASH2-containing H3K4 methyltransferase complex to methylate histone tail peptides containing H3R2me2a. Like that of the COMPASS complex in budding yeast, the activity of the ASH2-containing methyltransferase complex toward H3K4 was abrogated in the presence of H3R2me2a (**Fig. 1b**). In the human ASH2-containing H3K4 methyltransferase complex, the WDR5 protein subunit contributes to recognition of the H3K4me2 modification via its WD40 domain and may promote enzymatic conversion of H3K4me2 to H3K4me3 (ref. 13). Just as methylation recognition by the PHD-containing Spp1 protein in budding yeast is blocked by H3R2me2a, H3R2me2a also blocks recognition of H3K4me2 and H3K4me3 by the WD40 domain of WDR5. Previous structural analysis of WDR5 has shown that the H3R2 side chain binds deep within the core of the WD40 domain and is essential for H3K4 recognition[14–16]. In agreement with the inhibitory effects of H3R2me2a on recognition of histone tails *in vitro* by WDR5, ChIP analysis reveals a clear negative correlation between binding of H3K4 methyltransferase complex components and the presence of H3R2me2a at genes *in vivo*[4].

Now that H3R2me2a is known to be an important and widespread repressive histone modification, it will be essential to characterize H3R2me2a methyltransferase enzymes and discover how they are enzymatically regulated and targeted to chromatin. In budding yeast, production of H3R2me2a is probably catalyzed by an uncharacterized methyltransferase enzyme, as deletion of the three known arginine methyltransferases (Rmt1, Rmt2 and Hsl7) does not affect H3R2me2a levels[3]. In mammals, it seems that deposition of H3R2me2a may be catalyzed by either Prmt4 or Prmt6, depending on the cell type. Prmt4 has defined roles in histone arginine methylation during gene activation mediated by nuclear receptors, but Prmt6 has previously been shown to methylate non-histone substrates, including DNA polymerase β, HMGA1 and the human immunodeficiency virus TAT protein[17–19].

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Careful biochemical analysis of the H3R2 methylation activities of these Prmt enzymes will help to clarify the role of each in placing the H3R2me2a modification. Interestingly, Guccione *et al.*[4] observed that H3K4me3 can inhibit deposition of H3R2me2a by PRMT6, suggesting that active regions of chromatin that contain H3K4me3 may also function as a barrier to H3R2me2a (**Fig. 1b**). The recent observation that H3K4me3 is more widely distributed than previously appreciated, over both actively transcribed and poised promoter regions, suggests that mammalian genes may also rely on H3K4me3 to occlude repressive H3R2me2a from regulatory regions[20] (**Fig. 1b**). Further functional analysis of these enzymes will be required to define more precisely their contributions to H3R2me2a placement.

Kirmizis *et al.*[3] demonstrate in budding yeast that inducing expression of a silenced gene leads to a loss of H3R2me2a and the appearance of H3K4me3. This observation suggests that H3R2me2a may be dynamically regulated during the gene-activation process. In these experiments, chromatin modifications were analyzed 24 hours after induction, making it difficult to determine whether the loss of H3R2me2a was an active process or simply the result of the arginine methyltransferase enzyme being occluded from the 5′ end of the gene after DNA replication. Temporal analysis of H3R2 methylation status during gene activation will provide important information regarding whether active processes including histone replacement or demethylation are involved in dynamically regulating gene activation.

The PHD, WD40, chromo- and tudor domains have been shown to bind H3K4-methylated histone tails[7,14–16,21,22]. Atomic structures for all four of these protein domains in complex with H3K4-methylated peptides have been solved. In each instance, the H3R2 side chain is an essential feature contributing to protein interaction. It is now known that the CHD1 chromodomain[21], the WDR5 WD40 domain and the Spp1 PHD domain bind less efficiently to H3K4-methylated peptides when the peptides also contain H3R2me2a. This observation raises the interesting possibility that effector proteins that bind methylated H3K4 may be limited to regions of the genome that lack H3R2me2a. Notably, a subset of PHD domains has recently been identified that recognize H3K4 specifically in the unmethylated state[23,24]. The atomic structure of the unmethylated H3K4–binding PHD domain of the BHC80 protein in complex with a histone H3 peptide has recently been solved[23]. In contrast to recognition of methylated H3K4–binding PHD domains, molecular recognition of unmethylated H3K4 by BHC80 does not involve direct contacts between the H3R2 side chain and the PHD domain. BHC80 is a component of the LSD1 H3K4 demethylase complex and contributes to LSD1 targeting. H3R2-independent H3K4 recognition by BHC80 is intriguing, as this feature may permit the LSD1 complex to counteract H3K4 methylation in chromatin that contains H3R2me2a. Functional characterization of the BHC80 PHD domain will be required to determine whether H3R2me2a is compatible with chromatin recognition by the LSD1 complex. In addition, it will be interesting to determine whether the enzymatic domain of LSD1 can catalyze removal of methyl groups from methylated H3K4 substrates that also contain H3R2me2a.

The discovery that H3R2me2a helps to maintain the silent chromatin state and inhibit H3K4 methyltransferases offers new insight into the mechanisms by which active and repressed chromatin states are dictated and maintained. As with all exciting new advances, this work prompts additional important questions. How are enzymes that place H3R2me2a targeted to silenced regions of chromatin? Is H3R2me2a, once placed, also dynamically regulated? How does H3R2me2a function in combination with other post-translational modifications? Given the pace at which understanding of the histone-methylation system is advancing, answers to many of these questions should be forthcoming.

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