Acetylation-mediated histone H3 accessibility by chromatin modifiers

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Histone PTM "crosstalk" governs H3K4 methylation

Histone post-translational modification (PTM) plays a central role in gene regulation. Select **PTMs combine** to establish closed (refractory to transcription) or open (permissive to transcription) chromatin landscapes through **mediating DNA compaction** and **dictating which factors are recruited to or excluded from a region of chromatin**. Histone PTMs partake in **crosstalk**, where **one PTM influences the deposition or removal of another**. One mechanism of crosstalk takes place when a PTM(s) indirectly **promotes access** to another modifiable residue, "poising" it for modification. Previous studies of the H3K4 methyltransferase **MLL1 (KMT2A)** revealed that an **acetylation-mediated chromatin "switch" governs H3K4 methylation** read-write capability [1].

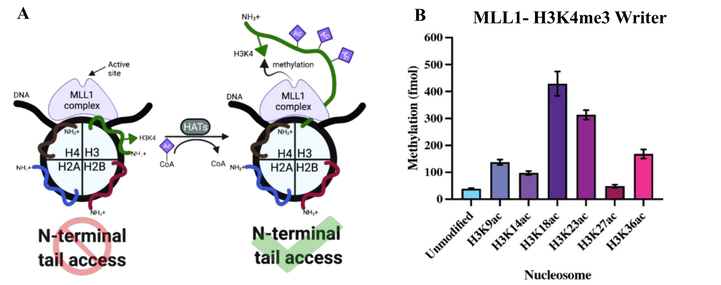

Figure 1. An acetylation-mediated chromatin switch governs H3K4 methylation read-write capability [1]. (A) Model for H3ac-stimulated H3K4 methylation by MLL1 (KMT2A). (B) *In vitro* methylation assays with recombinant MLL1 and differentially acetylated nucleosomes reveal a striking increase in MLL1-mediated H3K4me3 on nucleosomes bearing H3K18ac and H3K23ac.

Here, we expand on this work in asking a central question:

How does H3 acetylation contribute to the accessibility of other modifiable residues by different methyltransferases and chromatin modifying enzymes?

Methods: *in vitro* methylation assays

Each histone lysine methyltransferase that has been identified and described to date share a common cofactor: **S-adenosyl-L-methionine (SAM)**. When analyzing methylation *in vitro*, one can leverage this in utilizing **SAM containing a radioactive methyl group** in methylation assays, where histones or nucleosomes are incubated with recombinant methyltransferase and the **exchange of the radioactive methyl group to the substrate** is monitored, allowing for **quantitative analysis** of enzymatic activity and comparison of activity across different substrates.

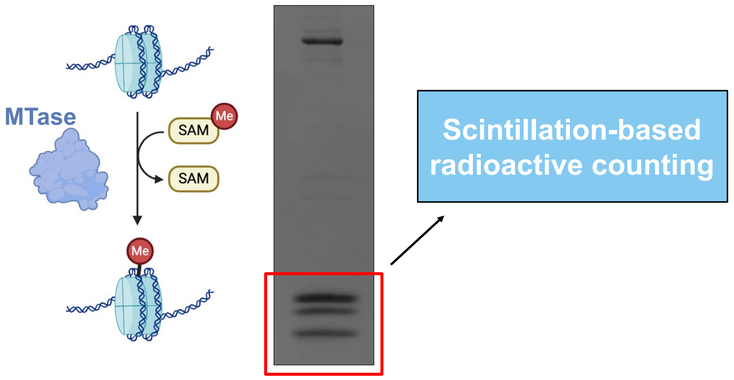

Figure 2. Schematic of the *in vitro* radioactive methylation assay. Tritiated (^{3}H) S-adenosyl-L-methionine (SAM) – the shared methyl donor cofactor amongst histone lysine methyltransferases – is incubated with recombinant methyltransferase and nucleosome. Following quenching, products are separated by SDS-PAGE, histone bands are excised and subjected to scintillation-based radioactive counting as a means of quantifying the exchange of the ^{3}H-methyl from SAM to the nucleosome.

Hypothesis

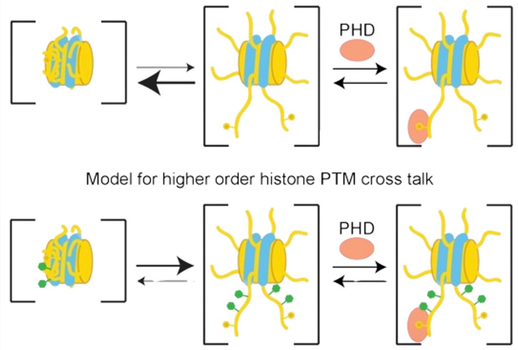

Figure 3. H3 acetylation (green) favors an "open" nucleosome conformation [2]. Nucleosomes exist in a dynamic state, ranging from closed (left) to open (middle). H3ac tips the balance to open, increasing accessibility to a modified or modifiable residue (right).

Because acetylation has been shown to increase nucleosomal accessibility to reader enzymes, we hypothesize that this effect would also extend to writer enzymes that modify H3 N-terminal tail residues.

H3K27ac stimulates H3K36me by SETD2 and NSD2

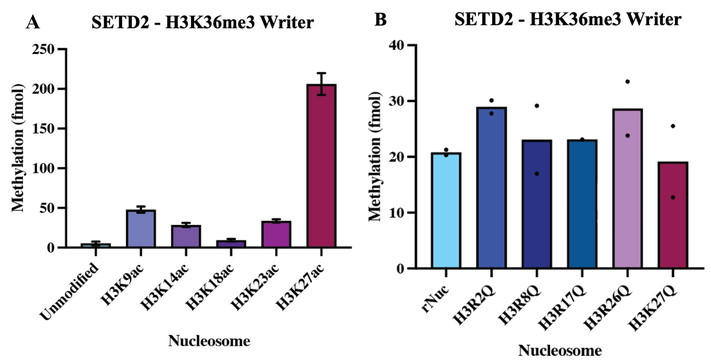

Figure 4. H3K27ac stimulates SETD2-mediated H3K36me3. (A) *In vitro* methylation assays with recombinant SETD2 and the designated nucleosomes (*EpiCypher*) reveal stimulation of SETD2-mediated H3K36me3 by H3K27ac. (B) *In vitro* methylation assays with glutamine mutant nucleosomes do not recapitulate the level of SETD2 stimulation.

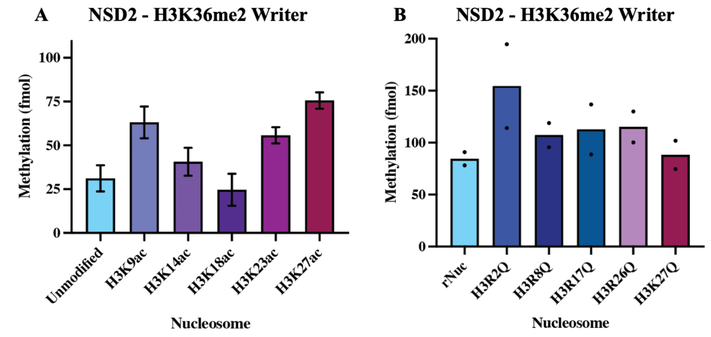

Figure 5. H3K27ac stimulates NSD2-mediated H3K36me2. (A) *In vitro* methylation assays with recombinant NSD2 and the designated nucleosomes (*EpiCypher*) reveal stimulation of NSD2-mediated H3K36me2 by H3K27ac. (B) *In vitro* methylation assays with glutamine mutant nucleosomes do not reproduce the level of NSD2 stimulation.

Other methyltransferases are insensitive to H3ac

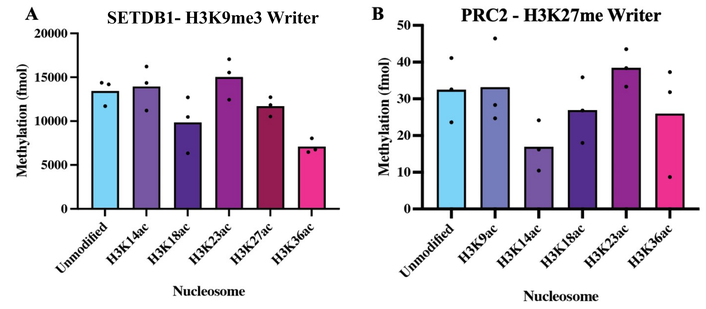

Figure 6. Other histone methyltransferases are largely insensitive to H3ac. *In vitro* methylation assays with (A) SETDB1 (H3K9me3 writer *in vivo*) & (B) PRC2 core complex (H3K27me1/2/3 writer *in vivo*) reveal a relative insensitivity of histone lysine methyltransferases targeting N-terminal H3 tail residues to H3ac.

Haspin-mediated H3T3ph is insensitive to H3ac *in vitro*

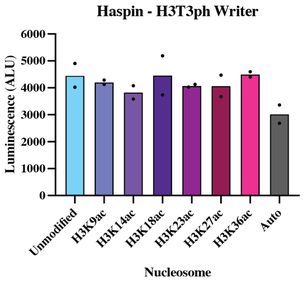

Figure 7. Haspin-mediated H3T3 phosphorylation is insensitive to H3 acetylation. *In vitro* luminescence-based kinase assays with recombinant Haspin kinase domain and the designated nucleosomes (*EpiCypher*) reveal minimal influence of H3ac on Haspin-mediated H3T3ph. Auto: autophosphorylation, no substrate (*in vitro,* Haspin autophosphorylates and assay cannot distinguish nucleosome phosphorylation from this).

Conclusions & Future Directions

1. **H3K27ac** stimulates **SETD2- and NSD2-mediated H3K36 methylation** *in vitro*
2. This effect **does not** extend to methyltransferases targeting other N-terminal H3 tail residues like H3K9 or H3K27, nor a kinase targeting H3K4's "nearest neighbor" modifiable residue (H3T3).

Further mechanistic studies of how H3K27ac may selectively "poise" H3K36 for access by the SETD2 and NSD2 methyltransferases are warranted. Additional emphasis will be placed on determining whether H3ac-mediated histone accessibility contributes to the activities of enzymes performing chemistry distinct from lysine methylation analyzed here (i.e., lysine demethylation, arginine methylation, etc.).

References & Acknowledgments

[1] Jain, K., et al (2023). An acetylation-mediated chromatin switch governs H3K4 methylation read-write capability. *eLife*, **12**:e82596.

[2] Morrison, E. A., Bowerman, S., Sylvers, K. L., Wereszczynski, J., & Musselman, C. A. (2018). The conformation of the histone H3 tail inhibits association of the BPTF PHD finger with the nucleosome. *eLife* **7**:e31481.

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