The Histone Code Influences Binding of Important Transcriptional Regulator

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GMR Deyter

Chromatin, a highly complex and intricate macromolecule, is formed when DNA is wrapped around histone octamers, making the DNA highly inaccessible to proteins that regulate transcription. However, appropriate transcriptional regulation is essential for many cellular processes and gene expression changes can have drastic consequences for cell proliferation and survival. Researchers from several institutes including the Parkhurst and Groudine Labs from Basic Sciences reveal in a recent publication the intricate details of how the MLL5 transcription regulator binds to chromatin. Post-doctoral fellow Hector Rincon-Arano of the Hutchinson Center is co-first author on the paper along with Muzaffar Ali from the University of Colorado School of Medicine.

MLL5 is a tumor suppressor protein and is frequently deleted in cancer cells from patients with hematological malignancies. Additionally, MLL5 plays critical roles in hematopoiesis, the DNA damage response, and other cellular processes. However, the mechanisms by which MLL5 influences these processes remain largely elusive. Even more unclear is how MLL5 gets recruited to chromatin, which is necessary in order for it to fine-tune gene expression.

As a starting point, the authors mapped the genome-wide localization of MLL5 in a mouse myoblast cell line to ~15,000 genomic regions, revealing enrichment of MLL5 at promoter regions downstream of transcription start sites. The co-enrichment of MLL5 and RNA polymerase at promoters indicated to the authors that MLL5 function correlates with highly transcribed genomic regions.

Histones are subject to a variety of post-translational modifications, and activating histone marks including lysine methylation can recruit proteins involved in transcription activation. Ali *et al.* found that MLL5 occupied promoters that displayed histone H3 lysine 4 trimethylation (H3K4me3), suggesting that this histone modification might recruit MLL5. Moreover, MLL5 has a PHD domain that is an established methylated histone binding module. The co-authors in the Kutateladze laboratory (University of Colorado) crystallized and solved the structure of the MLL5 PHD domain with a H3K4me3 peptide to reveal key amino acids in MLL5 that mediate the interaction. Interestingly, these analyses revealed a non-canonical mechanism by which the MLL5

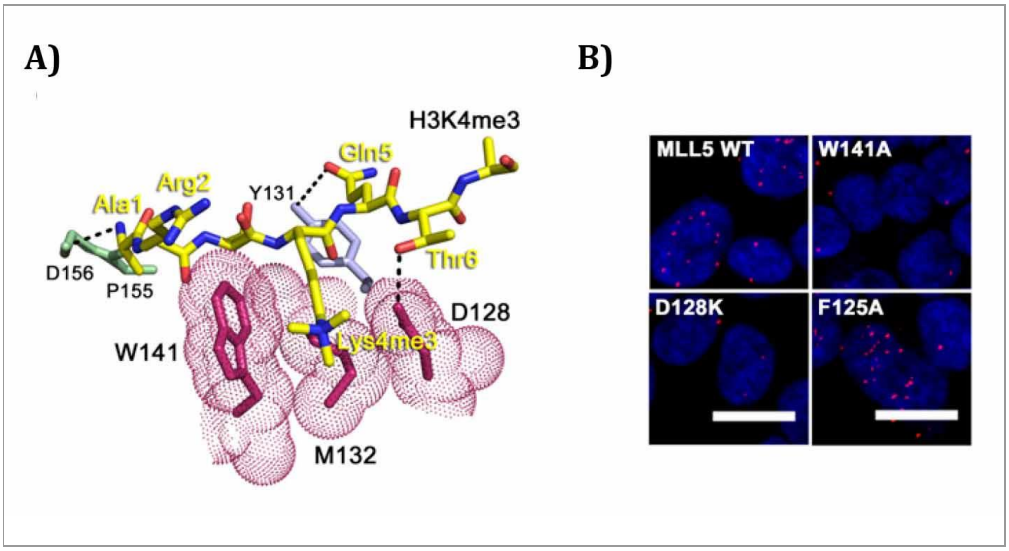
PHD domain binds trimethylated H3K4. The PHD domain of MLL5 uses only one aromatic residue to mediate H3K4me3 binding while the PHD domain of other proteins have two to four aromatic residues to mediate the binding.

Ali *et al.* co-stained cells with antibodies against H3K4me3 and wild-type or PHD domain-mutant MLL5 to determine if H3K4me3 promotes MLL5 binding via the PHD domain to chromatin *in vivo*. Secondary antibodies that fluoresce when the H3K4me3 and MLL5 signals are in close proximity (indicative of a protein-protein interaction) revealed multiple foci in wild-type MLL5 cells that were reduced in cells expressing MLL5 PHD-mutants that have decreased H3K4me3 binding (see figure). Since lysine 4 exists in a heavily post-translationally modified region of histone H3, the authors speculated that modifications of nearby H3 residues might disrupt the H3/MLL5 interaction. Indeed, they found that phosphorylation of histone H3 at Thr3 (H3T3ph) or Thr6 (H3T6ph) inhibited the H3K4me3/MLL5 interaction with *in vitro* protein binding assays. H3T3ph and H3T6ph occur during mitosis, and the authors found that MLL5 did not localize to mitotic chromosomes.

Lastly, the authors sought to determine whether the budding yeast and fly orthologs of MLL5 were similarly recruited to H3K4me3 chromatin and if H3T3ph or H3T6ph-promoted dissociation of MLL5 from chromatin was a conserved phenomenon. Strikingly, they found that the orthologous MLL5 proteins from these lower eukaryotes were similarly regulated, binding with high affinity to H3K4me3 and, in *Drosophila* cells, being excluded from chromatin containing the H3T3ph and H3T6ph marks.

Altogether, this manuscript makes a significant contribution toward understanding how an important transcriptional regulator is recruited to and excluded from chromatin. Part of the study's power lies in the strength and breadth of the techniques used, from traditional biochemical approaches to large-scale genomic analyses, using multiple model organisms. The precise role of MLL5 in regulating transcription remains to be determined, and the work by Ali and colleagues has revealed many clues about how and when MLL5 is recruited to chromatin to perform its essential functions.

Ali M, Rincón-Arano H, Zhao W, Rothbart SB, Tong Q, Parkhurst SM, Strahl BD, Deng LW, Groudine M, Kutateladze TG. 2013. Molecular basis for chromatin binding and regulation of MLL5. *PNAS* 110(28):11296-301.



Adapted from the manuscript

The PHD domain of MLL5 recruits MLL5 to chromatin. A) Diagram of important MLL5 residues (purple) that interact with specific histone H3 residues. The H3K4me3 binding residues of MLL5 include W141, M132, and D128. B) Cells expressing the indicated MLL5 proteins are stained to visualize the MLL5/H3K4me3 interaction (red foci) and DNA (blue). MLL5 mutants that were not expected to interact with histone H3 (as predicted by the crystal structure) did not bind robustly to H4K4me3 in vivo (W141A and D128K) while mutation of a neighboring residue of MLL5 (F125A) did not impact the interaction.