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RESEARCH HIGHLIGHT

Recognizing methylated histone variant H3.3 to prevent tumors

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Regulatory information stored in modified histones is functionally translated by effector proteins ('readers'), which identify the histone mark to determine the specificity of the response. A recent study identifying the tumor suppressor protein ZMYND11 as an exclusive reader of methylated histone variant H3.3, throws light on the role of transcription regulation in suppressing tumors.

Histone marks adorning chromatin function to maintain chromatin structure in either an open or closed conformation [1]. This structural modulation is necessary to regulate gene expression and other cellular processes that need access to DNA. How do these marks achieve their intended functions? In some cases, the marks themselves affect DNA-histone interactions (e.g., histone acetylation). In addition, many modifications act as tags that recruit specific protein factors or complexes involved in modulating chromatin structure [2]. Histone marks are 'read' and bound by chromatin remodeling complexes through highly conserved recognition domains, resulting in localized function at specific regions of the genome. Active readers 'open' chromatin by removing nucleosomes either completely or in parts, allowing access to DNA. This open chromatin is amenable to transcription by RNA polymerase. But unconstrained transcription is detrimental to the cell, thus the histones that are lost during transcription are replaced by new ones through a process called histone exchange.

Recent studies in yeast have elucidated how the marks and readers regulate the process of histone exchange during transcription. The H3K36 trimethyl mark (H3K36me3) is added to histones over gene bodies during the process of transcription by the RNA polymerase II (RNAPII)-associated enzyme, Set2 (Figure 1A-a). This mark oversees the resetting of the original chromatin structure after the passage of RNAPII by three different processes. First, it prevents replacement of new histones over gene bodies by excluding factors that promote histone exchange (histone chaperones, e.g., Asf1) (Figure 1A-b), ensuring the maintenance of the original nucleosomes and their associated marks [3]. It also recruits readers that (i) space the nucleosomes close to one another, avoiding the occurrence of free DNA (chromatin remodelers, Isw1b and Chd1) (Figure 1A-c) [4], and (ii) remove acetyl marks [5], preventing further destabilization of chromatin structure (histone deacetylase complex, Rpd3S) (Figure 1A-d). However, the picture gets complicated in higher eukaryotes where transcription entails the replacement of the original histones by the histone variant H3.3 [6]. Thus, promoters and coding regions of active genes are enriched for H3.3. In addition, this variant is also enriched for K36me3 over gene bodies [7], on account of the co-transcriptional addition of this mark. These observations bring up interesting questions as to what functional advantage does tagging the exchange-specific H3.3 variant with H3K36me3 give higher eukaryotes? Is the molecular mechanism of H3K36me3-mediated suppression of histone exchange restricted to yeast alone?

A recent study published by Shi and colleagues in *Nature* [8] begins to answer these questions. In order to functionally characterize the mammalian tumor suppressor protein ZMYND11, the authors focused on its role in regulating chromatin due to the occurrence of conserved protein domains that recognize different histone marks. Using peptide arrays and peptide pull-down assays, they concluded that ZMYND11 recognized the H3K36me3 peptides using a combination of three reader domains: a Plant Homeo Domain (PHD), a bromodomain and a PWWP domain (Figure 1B). Deletion of any of these domains, particularly the PWWP domain and bromodomain, abrogated the ability of ZMYND11 to bind the modified peptide. This is unusual as bromodomains normally bind to acetylated lysine residues, and the PWWP domain alone is sufficient to bind to H3K36me3. Is this domain combination utilized to recognize a more specific target? The authors answered this by analyzing the co-crystal structure of the modified peptide with the ZMYND11 interaction domain. Interestingly, the authors identified a novel zinc finger domain between the bromodomain and PWWP domain that bound the S31 residue of the histone peptide (Figure 1B). This residue is one of four amino acids in H3.3 that distinguishes it from the replication-specific histone variant H3.1 [9]. Indeed, the authors found that ZMYND11 was able to precisely identify and bind the K36 methylated H3.3

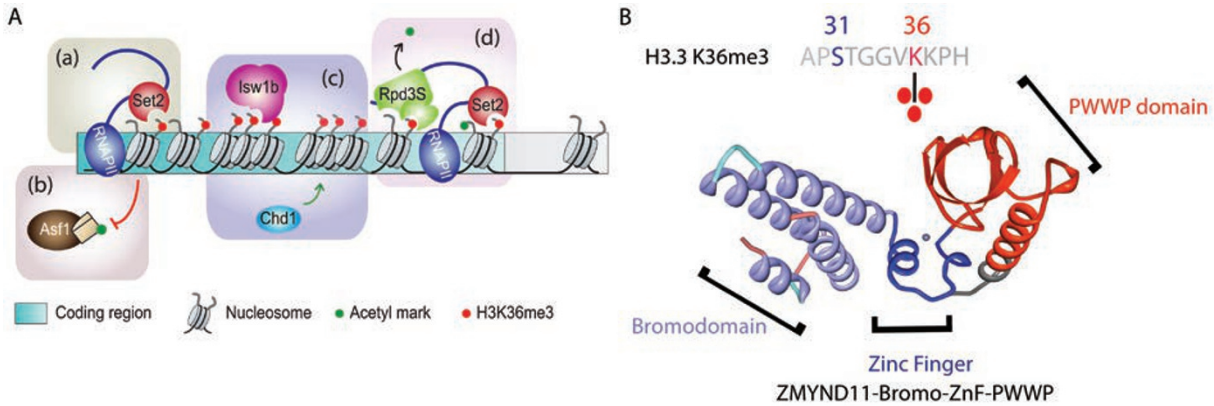

Figure 1 (A) Role of H3K36me3 in resetting chromatin. (a) Co-transcriptional addition of H3K36me3 by the lysine methyltransferase Set2 requires association with the elongating polymerase (RNAPII). (b) This mark impedes the ability of histone chaperones (Asf1) to replace existing nucleosomes from the soluble pool. (c) H3K36me3 recruits the chromatin remodeling complex, Isw1b, to coding regions where it acts with Chd1 to maintain chromatin structure. (d) Additionally, H3K36me activates the Rpd3S histone deacetylase complex, resulting in removal of acetyl marks from chromatin over coding regions thereby maintaining a closed chromatin structure. **(B)** Structure of ZMYND11 interaction domain with H3.3K36me3. Crystal structure of the ZMYND11 Bromodomain-Zinc Finger-PWWP domain fragment (ZMYND11-Bromo-ZnF-PWWP) with each domain color-coded to match with the respective interaction site on the H3.3K36me3 peptide (aa 29-39) shown here. Interestingly, the bromodomain does not contribute additional interactions with the N-terminal tail of the H3.3 peptide. The ribbon model of the protein was prepared using the PDB file 4N4I on the Protein Workshop viewer (PDB) [10].

in vivo. Thus, ZMYND11 uses an uncommon combination of histone reader modules to bind exclusively to the modified histone variant H3.3K36me3.

Employing whole-genome chromatin immunoprecipitation sequencing (ChIP-Seq), Shi and colleagues showed that ZMYND11 localizes to gene bodies enriched for both H3.3 and K36me3. Thus ZMYND11 function is aimed toward genomic regions having H3.3K36me3. But, what functional role does this reader play in transcription? Based on oncogene activation upon depletion of ZMYND11 in mammalian cell lines and its localization over gene bodies, the authors proposed a likely role in transcription elongation. The crucial question is whether this function of ZMYND11 required for its role as a tumor suppressor? The authors carried out mouse xenograft studies by injecting breast cancer cell lines expressing either the wild-type ZMYND11 protein or mutants that hamper its ability to bind to the H3.3K36me3 mark. Interestingly, while wild-type ZMYND11 expression prevented tumor formation in mice, the mutant proteins weakened its role as a tumor suppressor. The authors extended this study to analysis of breast cancer patients' expression profiling database, and they conclude that loss of ZMYND11 expression correlated with lower levels of survival. Therefore, the tumor suppressor function of ZMYND11 is associated with its ability to read H3.3K36me3 and modulate transcription elongation.

The cell utilizes various combinations from a repertoire of histone marks and variants that have evolved towards specific cellular functions. Reader recognition of histone marks ensures that effector protein localizes to specific genomic locations. Thus, specific identification of H3.3K36me3 by ZMYND11 ensures that it is targeted to tumor suppressor genes where it regulates productive elongation. Further work is required to answer whether ZMYND11 functions by resetting chromatin. However, the identification of ZYMD11 opens up the possibility that other variants and modification-specific readers exist to fine-tune responses based on histone marks.

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