Editorial

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Epigenomics

H2A.Z acetylation and transcription: ready, steady, go!

"...studying the incorporation of H2A.Z as a whole without considering the PTMs, is insufficient to understand the mechanism of action of this histone variant."

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In the cell nucleus, genomic DNA is packaged into chromatin, which consists of basic repeat units, called nucleosomes, which contain approximately 146 bp of DNA wrapped around an octamer of core histones (two molecules of each histone H2A, H2B, H3 and H4). The compaction of chromatin is a highly regulated and dynamic process and restricts the access of cellular machinery to DNA. Chemical modifications of the core histone tails and incorporation of histone variants act to modulate the chromatin structure, modifying the histone–DNA and histone–histone interactions. H2A.Z is an evolutionary conserved histone variant of the canonical H2A family, which shares approximately 60% amino-acid sequence. H2A.Z is essential for viability in many organisms, including mammals suggesting a unique and important role [1]. In fact H2A.Z has been implicated in many diverse and potentially contrasting functions such as gene transcription, nucleosome turnover, DNA repair, heterochromatin boundaries, chromosome segregation, progression through the cell cycle, suppression of antisense RNAs, embryonic stem cell (ESC) differentiation and antagonizing DNA methylation [1]. In particular several reports highlight potential conflicting roles of H2A.Z nucleosome occupancy in the regulation of gene activity, where H2A.Z has been implicated in active, poised or inactive gene expression [2–4]. A key question, therefore, is how can this variant have such a wide variety of functions, and potentially perform opposite roles?

Over the last 15 years, H2A.Z potential contradictory roles in gene transcription have been widely studied and it is now clear that there are multiple factors that affect H2A.Z repressive/poise/active roles [5]. One of these factors is H2A.Z post-translation modification (PTM) (reviewed in [6]). H2A.Z lysines can be either acetylated (K4, K7 and K11) [7,8] or methylated (K4 and K7) [9] at the N-terminal. In addition, three specific lysines (K120, K121 and K125) at the C-terminal can be monoubiquitinated [10]. Methylation of lysines 4 and 7 are required for ESC self-renewal and has been associated with negative regulation of gene expression [9]. Monoubiquitilated H2A.Z is found at facultative heterochromatin and is associated with repressed transcription [10,11], while acetylation is a mark of active chromatin [7,12–14] and confers nucleosome destabilization and an open conformation [8]. Interestingly, comodification of H2A.Z with both acetylation and monoubiquitination, is enriched at bivalent chromatin in ESCs [15].

Despite the reported existence and potential confounding functions of these PTMs, the scientific community primarily studies H2A.Z and its role in transcription blinded to PTM state of this histone variant, as the commonly used H2A.Z antibody in chromatin immunoprecipitation studies is agnostic to PTMs. In this editorial piece, we will summarize the scientific evidence supporting the thesis that PTMs, in particular

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acetylation of H2A.Z (H2A.Zac), and not unmodified H2A.Z, are correlated and functionally involved with active gene transcription.

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Acetylation is the best characterized modification of H2A.Z and plays a key role in transcriptional activation. In yeast, H2A.Z acetylation levels correlate with global gene activity [7], and it is required for galactose-dependent gene induction [12]. Similarly in chicken cells, the hyperacetylated form of H2A.Z is enriched at the 5′ end of active genes but is absent from inactive genes [13]. In humans, there are a number of studies that associate gene expression with H2A.Zac, with implications in cell differentiation [15,16] and cancer [14,17–19]. In fact, we have previously reported that H2A.Zac is present at promoters of highly expressed genes in normal and prostate cancer cell lines. Moreover, deregulation of gene expression in cancer is associated with H2A.Zac/H2A.Z remodeling, where silent genes become active by lose of underacetylated H2A.Z and gain of acetylated H2A.Z nucleosomes at gene promoters [14]. In accordance with our previous study, others have further reported the enrichment of promoter-associated H2A.Zac nucleosomes and active gene transcription. For example, in p53 negative breast cancer cells, depletion of H2A.Z is not enough to trigger p21 transcription but needs to be accompanied by an increase in promoter H2A.Zac levels [19]. In addition, the expression of the oncogene cyclin D1 requires eviction of non-acetylated H2A.Z and acetylation of the remaining H2A.Z-containing nucleosomes at the promoter and enhancer in estrogen receptor (ER) positive MCF-7 [18] and ER-negative MDA-MB231 [17] cell lines. In LNCaP prostate cancer cells, H2A.Z levels are reduced at both promoters and enhancers of the androgen-responsive genes *KLK3* and *KLK2* genes upon gene activation after androgen treatment [11], while the H2A.Zac/H2A.Z ratio is increased at KLK3 promoter and enhancer regions [20]. Moreover, genome-wide studies in ESCs show that H2A.Zac is enriched at poised and active promoters but not at stably repressed loci [15]. In particular, as transcription activity increases, total H2A.Z decreases at ESC promoters, but an increasing proportion of the variant becomes acetylated [15]. Together these studies suggest that a cross-talk between the total levels of H2A.Z and its acetylation status at gene promoters will dictate the transcriptional fate. Therefore, the lack of analysis of H2A.Z acetylation modification may lead to misconceptions about the actual role of H2A.Z in gene transcription.

H2A.Z is also enriched at enhancer regions [2,21] and notably H2A.Z is reported to be also acetylated at enhancers in ESCs [15,21]. The presence of H2A.Zac at enhancers opens new avenues for understanding how H2A.Z regulates gene transcription. In fact a recent publication [22] shows that H2A.Z is essential for ER enhancer activity, as it is required for RNA polymerase II (Pol II) recruitment, enhancer RNA transcription and enhancer–promoter interactions. Considering the previous studies in breast cancer cell lines where the H2A.Zac/H2A.Z ratio in promoters and enhancers was the determinant factor to induce gene transcription [17,18], it is highly likely that the functions attributed here to H2A.Z in enhancer activity could be caused by the acetylation status. We hypothesize that H2A.Zac is a potential mark of inducible genes, where H2A.Zac nucleosomes provide a more open conformation 'ready' state, which facilitates the binding of transcriptional factors and chromatin remodelers, that is, a 'steady' state, which in turn facilitates Pol II to initiate transcription, 'go' state. However, genome-wide and functional studies focuses on understanding the role of H2A.Zac at both enhancer and promoter regions are needed to further validate this hypothesis.

Even though it is now clear that H2A.Zac is a mark of active transcription, there are only a few studies reporting the functional link and phenotypic consequences of H2A.Z acetylation in the context of gene transcription. A recent study using a non-acetylable H2A.Z mutant investigated the importance of the acetylation in myogenesis. Ectopic expression of the H2A.Z mutant reduced chromatin accessibility to the MyoD promoter and inhibited MyoD expression. The lack of this transcription factor produced cell-cycle withdrawal defects, which ultimately resulted in a block of the myogenic differentiation process [16]. This study provides proof of principle that demonstrates the requirement of H2A.Zac is an essential element in the activation of the expression of master regulator genes. Thus, future studies should aim to dissect the specific role and mechanism of the acetylation of H2A.Z by generating site-specific mutations in normal development and disease.

The underlying molecular mechanism of how acetylation of H2A.Z influences gene activation remains poorly understood. One explanation could be due to changes to the nucleosome structure. The first (+1) nucleosome of the transcription start site is a major barrier to transcription *in vivo*, and H2A.Z incorporation reduces the nucleosomal barrier to Pol II [23]. In this model, H2A.Z–H2B dimer is more easily lost that the canonical dimer enhances the elongation of Pol II through nucleosomes. Accordingly, H2A.Z shows weaker interactions with H3–H4 compared

to H2A and the acetylation of the N-terminal region of H2A.Z may be responsible for this nucleosome destabilization [8]. On the other hand, histone modifications normally facilitate protein–protein interactions with effector proteins or readers, therefore, the N-terminal of H2A.Z could also represent the target for proteins implicated in transcription. The bromodomain containing transcriptional activator Brd2 preferentially associates with H2A.Z nucleosomes at active genes, and Brd2 recruitment is necessary for androgen receptor-regulated gene activation [24]. The fact that bromodomains are recruited to regulatory regions recognizing acetylated lysines, and H2A.Zac is present at androgen-dependant gene promoters [14], it is tempting to hypothesize that H2A.Zac facilitates the recruitment of Brd2 during gene activation. Another recent example of this association occurs in ESCs cells; Surface *et al.* have proposed a model where Brd2 is absent from bivalent genes enriched for monoubiquitylated H2A.Z, and removal of the ubiquitin group leads to Brd2 recruitment and gene activation of developmental genes [25]. H2A.Zac is present at bivalent and active promoters in ESCs cells and correlates with trancription activity [15], so Brd2 could be recruited at the promoter and bind to the acetylated lysines of H2A.Z. This data suggest that Brd2 could be a specific reader of H2A.Zac to ultimately activate gene transcription.

In conclusion, we purport that the key regulator of transcription is not the present or absence of H2A.Z but its acetylation status. Therefore, studying the incorporation of H2A.Z as a whole without considering the PTMs, is insufficient to understand the mechanism of action of this histone variant. We consider that we have only seen the 'tip of the iceberg' regarding the role of H2A.Z in gene regulation and more detailed studies on the different PTMs are needed to fully understand its complexity.

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