

Histone H4 lysine 16 acetylation breaks the genome's silence

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Abstract

Acetylation at histone H4 lysine 16 is involved in many cellular processes in organisms as diverse as yeast and humans. A recent biochemical study pinpoints this particular acetylation mark as a switch for changing chromatin from a repressive to a transcriptionally active state.

Eukaryotic DNA is packaged with proteins into substructures that are themselves packed into higher-order 30-nm fibers - the DNA-protein polymer called chromatin. The fundamental repeating unit in chromatin is the nucleosome, which consists of 146 bp of DNA wrapped around an octamer of histone proteins [1]. This 'core' octamer contains two histone H2A-H2B dimers, and a histone H3-H4 tetramer [1,2]. The compact architecture of chromatin naturally presents a significant barrier to all cellular events that require the underlying DNA and the accessibility of DNA is dynamically regulated through several distinct, but not mutually exclusive, mechanisms in order for transcription, DNA repair, replication and recombination to take place [3]. One of the most extensively studied mechanisms for altering chromatin structure is the posttranslational covalent modification of the histone amino-terminal tails. One particular modification, histone acetylation, has been known to correlate with transcriptional regulation for more than 40 years [4]. Histone acetylation generally makes chromatin accessible to the transcription-activating machinery, resulting in gene expression [5,6]. One exception, the acetylation of histone H4 at lysine 12, has been found in regions of silent heterochromatin; therefore histone acetylation is not always associated with active transcription [7,8]. Overall, the acetylation state of histones seems to regulate the interconversion of active and repressive chromatin structure [9], but the molecular mechanism underlying the effects of histone acetylation on the state of chromatin is still poorly understood.

Model choices

The effects of histone acetylation on transcription can be explained by two different but not mutually exclusive models [10]. In the first, the acetylation of histone tails at specific lysine residues may directly interfere with DNA-histone, histone-histone, and even internucleosomal interactions, resulting in chromatin decondensation and transcriptional activation. In the second, the acetylation mark serves as a signal for chromatin modifiers to bind and modulate transcription. A few years ago, Dorigo *et al.* [11] demonstrated that the histone H4 tail, especially amino acids 14-19, is essential for chromatin-fiber compaction. As the acetylation of histone H4 lysine 16 (H4 Lys16) is the only known modification in this region, it was reasonable to speculate that it affected the higher-order structure of chromatin.

A recent study by Shogren-Knaak *et al.* [12] now directly implicates acetylation of H4 Lys16 as the central switch for controlling higher-order chromatin structure. Using a chemical ligation technique, they generated histone H4 homogeneously acetylated at lysine 16. When assembled into nucleosomal arrays, the presence of acetylated H4 Lys16 inhibited the formation of higher-order 30-nm chromatin fibers as well as the fiber-fiber interactions. This relaxation of compacted chromatin structure is a unique effect of histone acetylation that is thought to have a role in chromatin decondensation and transcription activation. In addition to its effects on higher-order structure, acetylated H4 Lys16 also

inhibited the activity of the *Drosophila* chromatin assembly and remodeling enzyme ACF on the chromatin fiber. The work by Shogren-Knaak *et al.* [12] thus shows that acetylated H4 Lys16 not only contributes to the decondensation of compacted chromatin, but also that it can modulate the association of a specific remodeling enzyme with chromatin, providing further important details about how the state of chromatin is significantly changed by a single histone modification.

The recent findings by Shogren-Knaak *et al.* [12] regarding the specific structure of chromatin acetylated on H4 Lys16 correlates with other functions of H4 Lys16 acetylation studied in different organisms. Among various acetylatable lysines identified to date, histone H4 Lys16 is functionally unique in many ways. The special role of H4 Lys16 acetylation is clearly demonstrated in budding yeast (at silencing boundaries) [13,14], fruit flies (in dosage compensation) [15], and human cancer cells (in which H4 Lys16 acetylation is lost) [16].

Histone H4 Lys16 acetylation in yeast, flies and human cancer

In the budding yeast *Saccharomyces cerevisiae*, acetylation at H4 Lys16 is essential to maintain the proper boundaries of repression at all silent loci, including the *HML* and *HMR* mating-type loci, telomeres and rDNA arrays [17]. Transcriptionally repressed heterochromatin is hypoacetylated at H4 Lys16 as the result of the presence of Sir2, a histone deacetylase specific for H4 Lys16 [18]. Thus, acetylation of H4 Lys16 may prevent the ectopic spreading of heterochromatin. Indeed, the anti-silencing function of H4 Lys16 acetylation has been demonstrated by Kimura *et al.* [13] and Suka *et al.* [14] in studies that focused on telomeric regions. In yeast, the trimeric SAS complex is exclusively responsible for acetylating histone H4 at Lys16 [19]. Mutation at H4 Lys16, as well as deletion of *sas2*, the gene encoding the catalytic acetylase subunit in SAS, causes the Sir silencing proteins (Sir2, Sir3, and Sir4) to propagate from the telomeres farther into non-silenced euchromatic regions [13,14]. This phenomenon is consistent with microarray data showing that transcription of telomere-proximal genes was repressed in yeast carrying the mutation Lys16 to Arg in H4, or a *sas2* deletion. The repression is presumably due to deacetylation of histone tails by Sir2 [13,20]. Thus, it appears that competition between two functionally opposing histone-modification complexes sets up a dynamic acetylation state for H4 Lys16 that determines the heterochromatin-euchromatin boundary at telomeres. Several independent investigations have concluded that Lys16 is the most highly acetylated site in yeast histone H4 [21-24]. Specifically, the recent study by Shogren-Knaak *et al.* [12] provides a direct link between acetylated H4 Lys16 and chromatin structure in yeast.

Dosage compensation in male *Drosophila* presents another good example of how the specific acetylation of H4 Lys16

affects transcription and chromatin packaging. Male flies double the transcriptional activity of their single X chromosome to compensate for the fact that female flies carry two copies of the X chromosome [15]. This enhancement of transcription in the male X chromosome is achieved specifically by the male-specific lethal (MSL) complex, which contains a catalytic subunit, MOF, that acetylates histone H4 at Lys16 [25]. In female flies, the assembly of MSL is impaired, which results in hypoacetylation of H4 Lys16. In male flies, MSL binds to the X chromosome at hundreds of loci, and has a binding pattern similar to that of acetylated H4 Lys16 as confirmed by polytene chromosome immunostaining [26]. Once the MSL complex acetylates H4 Lys16, the X chromosome acquires a 'diffuse' appearance, which is reminiscent of decondensed chromatin and agrees with the recent yeast data [12,27]. Therefore, acetylated H4 Lys16 appears to 'open up' the *Drosophila* male X chromosome to make it more accessible to transcription, which is an important part of the dosage compensation mechanism in the fly.

In human cells, the acetylation state of H4 Lys16 has recently been noted as an epigenetic hallmark for certain types of cancers [16]. Abnormalities in DNA methylation status have been a major focus of cancer epigenetics for many years. Previous research has shown the association of DNA methylation with inaccessible chromatin and several histone-modification machineries [28,29]. The direct effect of global histone modifications in carcinogenesis, however, is still elusive. Work from Esteller's group [16,30] shows that specific monoacetylation of H4 Lys16 is lost in several human cancer cell lines and two primary tumors (lymphoma and colorectal adenocarcinoma). In contrast, there is no difference in the acetylation level at all the other lysine residues (Lys5, Lys8, and Lys12) in the amino-terminal tail of histone H4, suggesting a unique role for H4 Lys16 acetylation in preventing cell transformation. It is possible that H4 Lys16 acetylation at tumor suppressor genes protects them from being transcriptionally repressed in normal cells. Interestingly, the change in H4 Lys16 acetylation in cancer cells correlates well with the characteristic hypomethylation of repetitive DNA sequences. Therefore, the process of carcinogenesis includes epigenetic modifications at both the DNA and histone level. Still, the molecular basis of how the loss of H4 Lys16 acetylation results in cellular transformation is still poorly understood, but it may point to new uses for histone deacetylase inhibitors in cancer treatment. In addition, this specific acetylation marker could be used in the future as a tool [31] for cancer prediction and diagnosis.

H4 Lys16 acetylation is a unique feature that plays a vital role in the maintenance of chromatin structure [12]. To date, it is the only histone modification mark that can be directly linked to changes in chromatin folding [11,12,32]. Considering the variety of other types of histone modifications, it is unlikely that acetylation of H4 Lys16 acts alone in maintaining decondensed chromatin structure. Nevertheless, the

unique effect of acetylated H4 Lys16 on genome-wide chromatin dynamics makes it a vital epigenetic modification for the regulation of gene transcription.

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