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Histone H4 lysine 16 acetylated isoform synthesis opens new route to biophysical studies

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Abstract

Histone H4 lysine acetylation regulated by MOF (males absent on the first) was initially discovered as a dosage compensation epigenetic mark. Recent studies have revealed, however, that the epigenetic mark has a critical role in cellular function both during oogenesis as well as oncogenesis. Detailed molecular analysis of H4K16 isoforms and other posttranslational modified histones has been limited by the lack of means to prepare sufficient material for in vitro study. This paper describes an improved method to prepare acetylated H4K16 as well as other covalently modified histone H4 isoform for biophysical studies.

Keywords

Cancer; DNA damage response; H4K16ac; Transcription

The regulation of transcription as well as the DNA damage response involves the ATP-dependent remodeling of the DNA-histone octamer complex and the covalent modification of DNA and histones [1, 2]. One of the better studied among histone modifications is the acetylation of histone H4 at lysine 16 (H4K16ac). Loss of H4K16ac has been linked with certain tumor suppressor genes in cancer [3–5] and hypoacetylation has been associated with defective DNA repair [6–9] and premature senescence [10]. Moreover, H4K16ac is essential for embryogenesis and oncogenesis [11], which is consistent with the observation that this acetylation mark was shown to be instrumental in the maintenance of mouse embryonic stem cell self-renewal and pluripotency [12]. Interestingly, loss of H4K16ac in postmitotic mouse purkinje cells results in their loss and correlated with the backward walking along with other neurological defects [13]. Mouse T-cells with loss of H4K16ac did not differentiate and survive [14]. In normal cells, the acetylation of H4K16 has been correlated with promoter remodeling in order to activate gene transcription. It has also been implicated as a mechanism for decreasing the interaction between neighboring nucleosomes and generally loosening the level of compaction of chromatin [15, 16]. Recent studies have shown that a majority of H4K16ac sites are localized within genes, with only a relatively small fraction (~10%) found near promoters. Histone H4K16 acetylation has been found to

have limited correlation with transcription regulation of HEK293 cells (N. Horikoshi et al., in press), whereas H4K16ac has been demonstrated to have critical roles in regulating transcription in mouse embryonic stem cells [12]. Thus, H4K16ac-dependent transcription regulation is likely a cell type specific process.

To date, the vast majority of information generated on the epigenetic regulation of transcription has been of a biochemical nature: enumeration of the in vivo covalent modifications or of the remodeling steps that alter the relationship of DNA with the histone octamers, identification of the enzymes and complexes responsible for implementing these changes, and genomic mapping of single or clustered modifications coupled with bioinformatic analyses. Relatively recently, and to an increasing extent, biophysical tools have begun to be applied in an attempt to understand the mechanistic basis for epigenetic modification effects. Several in vitro experiments have demonstrated that the presence of H4K16ac in nucleosomes causes arrays to resist condensation [17–19] or weakens the self-association of single nucleosome particles [20]. Chromatin fibers reconstituted with chemically synthesized H4K16ac are more disordered [21]. For similar experiments, and for the plethora of novel experiments capable of exploring the biophysical consequences of epigenetic modifications, the indispensable requirement is the ability to reconstitute nucleosomes and chromatin fibers using purified, precisely modified histones.

Modified histones have been generated by native ligation or chemical modification. These procedures, however, often require considerable expertise, such as handling of hydrofluoric acid in order to generate the necessary thioesters. Technically more familiar to molecular biology laboratories is the method devised by J.W. Chin [22] that relies on replacing the target codon that needs to be modified with an amber codon that will be read by a tRNA preloaded with the sought after modified amino acid. Unfortunately, some modified histones and especially H4 acetylated at lysine 16, are expressed at extremely low levels in bacteria. In a Technical Brief published in the current issue, Kallappagoudar et al. [23] have circumvented this latter difficulty and have extended the general usefulness of the amber codon method for the synthesis of “designer” histones. This technology should provide a needed impetus to studies focused on the biophysical mechanisms by which histone modifications regulate chromatin structure and function.

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