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Biological functions of biotinylated histones⋆

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Abstract

Histones H1, H2A, H2B, H3 and H4 are DNA-binding proteins that mediate the folding of DNA into chromatin. Various posttranslational modifications of histones regulate processes such as transcription, replication and repair of DNA. Recently, a novel posttranslational modification has been identified: covalent binding of the vitamin biotin to lysine residues in histones, mediated by biotinidase and holocarboxylase synthetase. Here we describe a novel peptide-based technique, which was used to identify eight distinct biotinylation sites in histones H2A, H3 and H4. Biotinylation site-specific antibodies were generated to investigate biological functions of histone biotinylation. Evidence was provided that biotinylation of histones plays a role in cell proliferation, gene silencing and cellular response to DNA damage.

Keywords

Biotin; Chromatin; DNA damage; Epigenetics; Histones; Human

1. Chromatin structure

Chromatin is comprised primarily of DNA and DNA-binding proteins, that is, histone and nonhistone proteins. Histones are essential for the folding of DNA into chromatin [1]. Five major classes of histones have been identified in mammals: H1, H2A, H2B, H3 and H4. Histones consist of a globular domain and a more flexible amino terminus (histone “tail”). Lysine (K) and arginine (R) residues account for a significant fraction of the amino acids in histones, mediating a positive net charge of these proteins at physiological pH [1].

DNA and histones form repetitive nucleoprotein units, the nucleosomal core particle [1]. Each core particle consists of 146 base pairs of DNA wrapped around one H3–H3–H4–H4 tetramer and two H2A–H2B dimers. The binding of DNA to histones is of electrostatic nature and is mediated by the association of negatively charged phosphate groups of DNA with positively charged ε-amino groups (lysine moieties) and guanidino groups (arginine moieties) of histones. The DNA located between nucleosomal core particles is associated with histone H1.

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The amino terminal tail of histones protrudes from the nucleosomal surface; covalent modifications of this tail affect the structure of chromatin and form the basis for gene regulation, mitotic and meiotic chromosome condensation and DNA repair [1,2]. Histone tails are modified by covalent acetylation [3–5], methylation [1], phosphorylation [1], ubiquitination [1], poly(ADP-ribosylation) [6–8] and biotinylation (see further discussions) of ε-amino groups (lysine), guanidino groups (arginine), carboxyl groups (glutamate) and hydroxyl groups (serine, S). Multiple signaling pathways converge on histones to mediate covalent modifications of specific amino acid residues [9,10]. Site-specific modifications of histones have distinct functions; for example, dimethylation of K4 in histone H3 is associated with transcriptionally active DNA [11,12]. Modifications of histone tails considerably extend the information potential of the DNA code and gene regulation [12–14]. Modifications of histone tails may affect binding of chromatin-associated proteins, triggering cascades of downstream histone modifications. For example, methylation of R3 in histone H4 recruits the histone acetyl transferase Esa1 to yeast chromatin, leading to acetylation of K5 in histone H4 [12]. Histone modifications can influence each other in synergistic or antagonistic ways, mediating gene regulation. For example, phosphorylation of S10 inhibits methylation of K9 in histone H3, but is coupled with acetylation of K9 and K14 during mitogenic stimulation in mammalian cells [12]. Covalent modifications of histones can be reversed by a large variety of enzymatic processes [12].

2. Enzymatic biotinylation and debiotinylation of histones

Histones are modified by covalent attachment of the vitamin biotin. Hymes et al. [15,16] have proposed a reaction mechanism by which cleavage of biocytin (biotin-ε-lysine) by biotinidase leads to the formation of a biotinyl-thioester intermediate (cysteine-bound biotin) at or near the active site of biotinidase [2]. In the next step, the biotinyl moiety is transferred from the thioester to the ε-amino group of lysine in histones. Biocytin is generated in the breakdown of biotin-dependent carboxylases, which contain biotin linked to the ε-amino group of a lysine moiety [17].

Biotinidase belongs to the nitrilase superfamily of enzymes, which consists of 12 families of amidases, *N*-acyltransferases, and nitrilases [18]. Human biotinidase has been characterized at the gene level [19,20]. The 62 amino acid region that harbors the active site of biotinidase is highly conserved among various mammals and *Drosophila* [21]. Biotinidase is ubiquitous in mammalian cells and 26% of the cellular biotinidase activity is located in the nuclear fraction [22].

Subsequent to the elucidation of the biotinidase-mediated mechanism of histone biotinylation in vitro [15,16], biotinylated histones H1, H2A, H2B, H3 and H4 were detected in human lymphocytes in vivo [23]. Biotinylated histones were also detected in human lymphoma cells [24], small cell lung cancer cells [25], choriocarcinoma cells [26] and chicken erythrocytes [27]. These studies also suggested that biotinidase may not be the only enzyme-mediating histone biotinylation. For example, evidence was provided that biotinylation of histones increases in response to cell proliferation, whereas biotinidase activity was similar in nuclei from proliferating cells and quiescent controls [23]. Finally, Narang et al. [28] identified holocarboxylase synthetase as another enzyme that may catalyze biotinylation of histones.

Mechanisms mediating debiotinylation of histones are largely unknown. Recent studies suggested that biotinidase may catalyze both biotinylation and debiotinylation of histones [29]. Variables such as the microenvironment in chromatin, and posttranslational modifications and alternate splicing of biotinidase, might determine whether biotinidase acts as biotinyl histone transferase or histone debiotinylase [2].

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3. Identification of biotinylation sites

Biotinylation sites in human histones were identified by using synthetic peptides [30,31]. Briefly, this approach is based on the following analytical sequence: (i) short peptides (<20 amino acids in length) are synthesized chemically; the amino acid sequences in these peptides are based on the sequence in a given region of a given histone; (ii) peptides are incubated with biotinidase or holocarboxylase synthetase to conduct enzymatic biotinylation; (iii) peptides are resolved by electrophoresis; and (iv) biotin in peptides is probed using streptavidin peroxidase. Using this approach, the following biotinylation sites have been identified in human histones: K9, K13 and K129 in histone H2A (Y.C. Chew and J. Zempleni, unpublished observation), K4, K9 and K18 in histone H3 [32] and K8 and K12 in histone H4 [30]. Acetylation and phosphorylation of lysine and serine residues, respectively, decrease biotinylation of adjacent lysine residues [30,32]. In contrast, dimethylation of arginine residues enhances biotinylation of adjacent lysine residues [32].

4. Biological functions of histone biotinylation

Biotinylation of histones is a relatively new field of research; evidence of biological roles for biotinylation of histones is scarce. However, biotinylation of histones appears to participate in the following biological processes.

First, evidence was provided that biotinylation of histones increases in response to cell proliferation in human lymphocytes [23]. Biotinylation of histones increases early in the cell cycle (G1 phase) and remains increased during later phases (S, G2 and M phase) compared with quiescent controls; the increase is greater than fourfold. Fibroblasts from patients with holocarboxylase synthetase deficiency are severely deficient in histone biotinylation [28]. It remains to be determined whether this is associated with decreased proliferation rates. Note that these studies were conducted before specific biotinylation sites in histones were identified and before biotinylation site-specific antibodies became available. Thus, these studies did not allow pinpointing changes in specific biotinylation sites; rather, the global biotinylation of histones was quantified by using streptavidin or radiolabeled biotin. Recently, evidence emerged to suggest that biotinylation of distinct lysine residues in histone H4 changes at specific phases of the cell cycle [33].

Second, studies in chicken erythrocytes have provided circumstantial evidence that biotinylated histones are enriched in transcriptionally silent chromatin [27].

Third, biotinylation of histones might play a role in the cellular response to DNA damage [27,34]. If formation of thymine dimers is caused by exposure of lymphoid cells to UV light, the global biotinylation of histones increases [27]. If double-stranded DNA breaks are caused by exposure of lymphoid and choriocarcinoma cells to etoposide, biotinylation of K12 in histone H4 shows a rapid and transient decrease [34]. This is consistent with a role for histone biotinylation in signaling DNA damage. These studies suggest that distinct kinds of DNA damage cause unique changes in histone biotinylation. Currently, it is unknown whether biotinylation of histones is a mechanism leading to DNA repair or apoptosis.

5. Biotin supply

Effects of biotin supply on biotinylation of histones have been investigated in various human-derived cell lines [24–26]. In these studies, cell lines were cultured in media containing deficient, physiological and pharmacological concentrations of biotin for several weeks. Biotin concentrations in culture media had only a moderate impact on biotinylation of histones; in contrast, biotinylation of carboxylases correlated strongly with biotin concentrations in culture media [24–26]. The reader should note that even small changes in biotinylation of histones

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might be physiologically meaningful, given that these changes might affect other modifications of histones such as acetylation and methylation.

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