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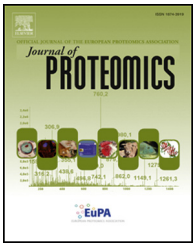

Identification of novel post-translational modifications in linker histones from chicken erythrocytes

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ABSTRACT

Chicken erythrocyte nuclei were digested with micrococcal nuclease and fractionated by centrifugation in low-salt buffer into soluble and insoluble fractions. Post-translational modifications of the purified linker histones of both fractions were analyzed by LC–ESI–MS/MS. All six histone H1 subtypes (H1.01, H1.02, H1.03, H1.10, H1.1L and H1.1R) and histone H5 were identified. Mass spectrometry analysis enabled the identification of a wide range of PTMs, including N^{α}-terminal acetylation, acetylation, formylation, phosphorylation and oxidation. A total of nine new modifications in chicken linker histones were mapped, most of them located in the N-terminal and globular domains. Relative quantification of the modified peptides showed that linker histone PTMs were differentially distributed among both chromatin fractions, suggesting their relevance in the regulation of chromatin structure. The analysis of our results combined with previously reported data for chicken and some mammalian species showed that most of the modified positions were conserved throughout evolution, highlighting their importance in specific linker histone functions and epigenetics.

Biological significance
Post-translational modifications of linker histones could have a role in the regulation of gene expression through the modulation of chromatin higher-order structure and chromatin remodeling. Finding new PTMs in linker histones is the first step to elucidate their role in the histone code. In this manuscript we report nine new post-translational modifications of the linker histones from chicken erythrocytes, one in H5 and eight in the H1 subtypes. Chromatin fractionated by centrifugation in low-salt buffer resulted in two fractions with different contents and compositions of linker histones and enriched in specific core histone PTMs. Of particular interest is the fact that linker histone PTMs were differentially distributed in both chromatin fractions, suggesting specific functions. Future studies are needed to establish the interplay between PTMs of linker and core histones in order to fully understand chromatin regulation. A protein sequence alignment summarizing the PTMs found to date in chicken, mouse, rat and humans showed that, while many of

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the modified positions were conserved between these species, the type of modification often varied depending on the species or the cellular type. This finding suggests an important role for the PTMs in the regulation of linker histone functions.

1. Introduction

The eukaryotic genome is tightly organized and packaged in a nucleoprotein complex called chromatin. The basic structural element of chromatin is the nucleosome, consisting of an octamer of core histones, two full turns of DNA (~166 bp), a linker histone molecule and a variable length of linker DNA. Linker histones are involved in the formation and stabilization of higher-order chromatin structures [1,2].

H1 has multiple isoforms. The histone H1 complement in chicken erythrocytes is composed of six different subtypes, named H1.01, H1.02, H1.03, H1.10, H1.1L and H1.1R [3]. In addition to the H1 subtypes, chicken erythrocytes contain histone H5, an avian-specific isoform [4]. H5 partially replaces histone H1 in mature erythrocytes, and is the most abundant subtype in these terminally differentiated cells [5]. Its function has been linked to the prevention of cell proliferation and chromatin condensation [6]. Somatic mammalian cells contain seven H1 subtypes, designated H1.1–H1.5, H1.0 and H1.10. H1.0 is thought to be mainly restricted to terminally differentiated cells, so it is considered the mammalian counterpart of H5 [7]. An oocyte-specific subtype, H1.8, and three male germ-line-specific subtypes, H1.6, H1.7 and H1.9, have also been identified [8].

Linker histones have three distinct domains: a short amino-terminal domain (NTD) (20 to 35 amino acids), a central globular domain (GD) (~80 amino acids) and a long carboxy-terminal domain (CTD) (~100 amino acids) [9]. The globular domain is primarily involved in DNA binding and very likely localizes H1 to the nucleosome. The terminal domains are intrinsically disordered regions, which are thought to be involved in binding non-histone chromatin proteins that regulate transcriptional activity [10]. The CTD is involved in chromatin condensation through binding and neutralization of the charge of the linker DNA [11]. This domain binds preferentially to SAR-DNA and also mediates protein–protein interactions with apoptotic nuclease DFF40 or heterochromatin protein HP1 [12–14]. The CTD shows a remarkable conformational flexibility; in particular, phosphorylation at specific sites by cyclin-dependent kinases (CDKs) greatly affects its DNA-bound structure and DNA condensing capacity [15].

A wide range of post-translational modifications have been identified in the N-terminal domain of core histones, including acetylation, methylation, phosphorylation and ubiquitination. They have been well characterized and are known to affect the contacts between nucleosomes and to be determinant for the binding and recruitment of proteins or protein complexes to the nucleosomes. The crosstalk between the different types of modifications at specific positions results in a complex network that has been involved in transcriptional regulation, DNA repair, RNA processing and signal transduction [16]. The post-translational modification of histones is an epigenetic mechanism that has been shown to be altered in disease [17]. Loss of acetylation at K26 and trimethylation at K20 of histone H4 have been associated with hypomethylation of DNA repetitive sequences, a characteristic of cancer cells [18]. Changes in histone modifications have also been detected in different cancer types, suggesting that different histone modifications can be used as biomarkers for early diagnosis and prognosis of cancer [19].

Linker histones are involved in chromatin structure and gene regulation. It is currently accepted that histone H1 could have a regulatory role in transcription through the modulation of chromatin higher-order structure or chromatin remodeling [20–23]. Thus, as in core histones, dynamic post-translational modifications may also play a role in the regulation of gene expression. In recent years, some effort has been made to characterize linker histone post-translational modifications in tissues and cellular lines of different organisms [7,24–30]. PTMs including phosphorylation, acetylation, methylation, formylation, ubiquitination, deamidation and citrullination have been identified in linker histones.

Some H1 modifications, like the methylation of K26 of H1.4 (H1.4K26me), have been associated with protein–protein interactions and the regulation of the binding of chromatin factors. Methylated K26 of H1.4 binds to HP1 via its chromodomain. This modification is part of a methyl–phos switch in which neighboring S27 phosphorylation reverts the effect of histone lysine methylation, inhibiting HP1 binding [31]. Interestingly, HP1 also binds H3K9m3, an epigenetic mark associated with heterochromatin, via its chromodomain. Since HP1 dimerizes via its chromoshadow domain, some authors have speculated that HP1 dimers could integrate this positional information, binding at the same time to H3K9me3 and H1.4K26me, in a manner important for chromatin compaction [32].

There is also increasing evidence of the role of epigenetic H1 modifications in cancer. In particular, alterations in H1 CDK-dependent phosphorylation have been associated with the progression of head and neck squamous-cell carcinomas and bladder cancer, suggesting that H1 phosphorylation has potential utility as a biomarker for cancer diagnosis and prognosis [33,34]. For these reasons, further data comprising new PTMs are necessary in order to characterize the modifications of linker histones in depth and their possible role in transcriptional regulation and disease.

In the present study, native chromatin from chicken erythrocytes was fractionated into soluble and insoluble fractions. Post-translational modifications in linker histones extracted from those two fractions were identified by ESI/LC–MS/MS, including N^{α}-acetylation, lysine acetylation, and formylation, and phosphorylation. Nine novel PTMs in chicken linker histones were identified. Relative quantification showed that linker histone PTMs were differentially distributed among both chromatin fractions. An analysis of the conservation of the post-translationally modified positions between avian and mammalian linker histones was also carried out.