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H1–nucleosome interactions and their functional implications☆

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ABSTRACT

Linker histones are three domain proteins and consist of a structured (globular) domain, flanked by two likely non-structured NH_2- and COOH-termini. The binding of the linker histones to the nucleosome was characterized by different methods in solution. Apparently, the globular domain interacts with the linker DNA and the nucleosome dyad, while the binding of the large and rich in lysines COOH-terminus results in "closing" the linker DNA of the nucleosome and the formation of the "stem" structure. What is the mode of binding of the linker histones within the chromatin fiber remains still elusive. Nonetheless, it is clear that linker histones are essential for both the assembly and maintenance of the condensed chromatin fiber. Interestingly, linker histones are post-translationally modified and how this affects both their binding to chromatin and functions is now beginning to emerge. In addition, linker histones are highly mobile *in vivo*, but not *in vitro*. No explanation of this finding is reported for the moment. The higher mobility of the linker histones should, however, have strong impact on their function. Linker histones plays an important role in gene expression regulation and other chromatin related process and their function is predominantly regulated by their posttranslational modifications. However, the detailed mechanism how the linker histones do function remains still not well understood despite numerous efforts. Here we will summarize and analyze the data on the linker histone binding to the nucleosome and the chromatin fiber and will discuss its functional consequences. This article is part of a Special Issue entitled: Histone H1, edited by Dr. Albert Jordan.

1. Introduction

The basic repeating unit of chromatin is the nucleosome, a complex of an octamer of the core histones (two of each H2A, H2B, H3 and H4), the linker histone (LH) and DNA of length varying between ~165 bp and 245 bp in both different organisms and cell types. The Nucleosomal Core Particle (**NCP**) is a nucleosomal substructure, formed by the histone octamer, around which 147 bp of DNA is wrapped in approximately 1.65 turns of left-handed helix [1]. The NCP has been structurally characterized to atomic resolution level [2–6]. A particle of 167 bp DNA in length (containing additional 10 bp DNA at each end of the core particle), the histone octamer and the linker histone (LH) is called **chromatosome** [7]. The DNA, interconnecting individual consecutive nucleosome cores in chromatin, is completing the structure of the **nucleosome**. Its length varies from 10 to 80 bp in different organism and cell types and is usually termed **linker DNA** (or spacer DNA) [1].

2. Linker histone structure and subtypes

Linker histones are the most variable of all histones. They are lysine rich and have three distinct domains; a structured central globular domain (GD) and apparently unstructured COOH- and NH_2-termini. Of note, the structured domain does not have a typical histone fold. Instead, it is organized in winged-helix pattern with three helices (α1 to α3), three loops (L1-L3) and two short beta sheets (B1 and B2) in hairpin [8,9] (Fig. 1).

The linker histone family is composed of at least 15 species, development stage- and tissue-specific isoforms [10–12], with eleven of them – H1.0, H1.1 (H1a), H1.2 (H1.b), H1.3 (H1c), H1.4 (H1d), H1.5 (H1e), H1t, H1T2, H1oo, H1LS1 and H1X – found in mammals (for linker histone nomenclature see [13]). Seven of the mammalian variants, H1.(1–5), H1.0 and H1X, were detected in somatic cells [13,14]. Although, H1.1 is specific for spleen, thymus and testis, and H1.0 is characteristic for terminally differentiated cells. A specific subtype, characteristic for avian and amphibian erythrocytes, is the linker histone H5 [15]. Distinct linker histone embryonic subtypes B4, H1oo and dBigH1 in amphibians, mouse and Drosophila respectively, were also identified and functionally analyzed [16–18]. A particular case is Hho1p, the linker histone in *Saccharomyces cerevisiae*, which has two globular domains [19]. *In vivo*,

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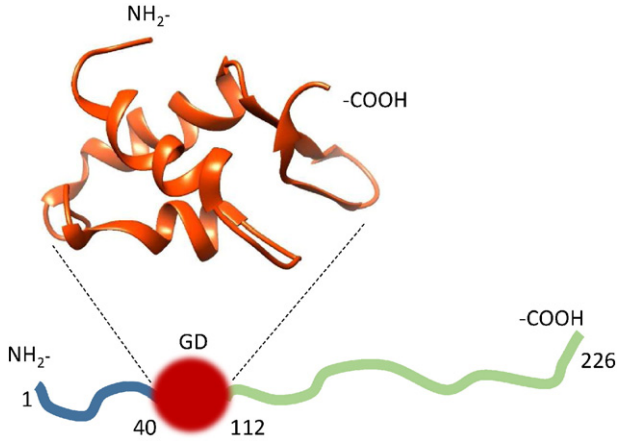

Fig. 1. Linker histone structure. Structured globular domain (GD) is flanked by short NH2 and long COOH termini, both unstructured. The indicated aa residue positions corresponds to H1.5. and GD structure is of chicken H1 (PDB ID: 1GHC).

the deposition of linker histones to nucleosome is assisted by dedicated chaperones. Individual somatic H1 subtypes differ in their expression pattern during development and differentiation [20], their ability to bind to and condense chromatin substrates [21] and their phosphorylation pattern during the cell cycle [22].

3. Linker histone chaperones

Linker histone chaperones are proteins, which specifically recognize linker histones and are involved in their deposition/removal from chromatin. The first protein that was suggested to have H1 chaperoning properties was nucleolin [23]. It was shown that the phosphorylated N-terminal domain of nucleolin has a strong affinity for histone H1, but not for the core histones [23]. Later on it was found that in Xenopus egg extract, the protein NAP-1 forms a complex with B4, the embryonic subtype of frog linker histone [24]. Detailed analysis identified it as a chaperone for B4. Interestingly, glutamylation of NAP-1 is likely to modulate both B4 dynamics and chromosome condensation [25]. More recent studies revealed that Template Activating Factor-I (TAF-I) behaved as a linker histone chaperone and is involved in linker histone dynamics [26]. TAF-1 was found specifically associated with H1 in mammalian somatic cell nuclei. In addition, nucleolar multifunctional protein nucleophosmin (NMP1) was identified as a new member of the linker histone chaperon family in humans [27]. NMP1 interacts with linker histone H1 through its first acidic stretch (residues 120–132) and was able to efficiently deposit H1 onto dinucleosomal templates *in vitro*. Many linker histone chaperones should exist. It is, however, unknown whether and how these chaperones differentiate between the different linker histone subtypes.

4. Linker histone binding to the nucleosome

Binding of linker histones to chromatin affects both the conformation of individual nucleosomes and the chromatin fiber folding. This is why a special attention was paid and numerous studies were carried out on the mode of linker histone interactions with chromatin.

4.1. Analysis of the linker histone–nucleosome interactions using natural templates

The early experiments using micrococcal nuclease digestion of native chromatin have revealed that the presence of linker histone extends the DNA protection pattern from 147 bp to about 166 bp, suggesting that H1 interacts with 10 bp of each linker DNA [7,28], and allowed the definition of "chromatosome". DNaseI footprinting of linker histones H5 and H1 on the nucleosome showed that the globular domain is protecting the dyad, while the NH2- and COOH termini reflect the protection at sites away from the dyad axis [29]. The "zig-zag "relaxed chromatin fiber structure observed at low ionic strength for H1-containing, but not for H1 depleted chromatin, illustrated the ability of H1 to set the exit angles of DNA from the chromatosome [30–32]. This determines, in turn, the angle between the flat faces of the nucleosomal disks and the axis of the fiber [33,34]. Trypsin digestion studies revealed that the globular domain of H1 was sufficient for maintaining this angle [34]. The use of a number of biophysical techniques showed that the long and lysine-rich COOH terminus of linker histones is required for chromatin fiber condensation (for a recent review, see [35]). To perform this function the linker histone COOH terminus is likely to be assisted by the NH_2-tail of H3, which is found to interact with the linker DNA [36]. Electron microscopy analysis suggested that the NH_2-tail of H1 plays a role in the localization of the globular domain of H1 [37]. The role of NH_2-tail in LH binding can be, however, more complex. Vyas and Brown [38] studied binding affinity of H1.0 and H1.3 using FRAP. When the N-terminal domains between H1.0 and H1.3 were swapped, H1.0 affinity changed to that of H1.3 and vice versa. Swapping COOH termini did not influence the affinities of studied H1 subtypes. This suggests that linker histone subtypes bind to chromatin in a specific manner determined by terminal domains.

4.2. In vitro studies on linker histone binding

In vitro reconstitution of nucleosomes by using purified components has revealed a detailed mapping of the H1–nucleosome interactions. In these experiments the use of specific DNA was determinant as it allows the reconstitution of well positioned nucleosomes. Initially, the natural sequence of the 5S ribosomal frog gene, which possesses a nucleosome positioning signal, was used for reconstitution (reviewed in [39]). The binding of the linker histones to these nucleosomes was analyzed by different techniques, including site directed both crosslinking and cleavage of DNA as well as hydroxyl-radical OH footprinting [40–42]. This has allowed the localization of the binding site of the linker histone globular domain in the nucleosome and to propose a model for the structure of the linker histone-bound nucleosome particle [43]. The linker DNA and H1-dependent reorganization of histone–DNA interactions was also studied. Of note, the binding of H1 was found to require a 5S nucleosome having only a single linker of length of 20 bp [42,44]. In addition, analysis of H1 binding to di-nucleosomal 5S templates was performed and the results were in general agreement with these of the mononucleosome [44]. These past studies suffered, however, from the relatively low strength positioning signal of the 5S DNA sequence. Indeed, the reconstituted nucleosomes exhibited multiple translational positions, which appeared to interfere with the mapping of the histone H1/nucleosome contacts [45]. In addition, these experiments used salt dialysis or direct binding to deposit H1 to the nucleosome, which may affect the localization of H1 on the nucleosome (discussed in [46]). These problems were overcome more recently by using both the physiologically relevant linker histone chaperone NAP-1 and the artificial 601 sequence with much stronger positioning signal. This has allowed for reconstitution of precisely positioned mono-, di-, and trinucleosomal templates and deposition of H1 or truncated mutants under physiological conditions [46]. Hydroxyl radical (OH) footprinting at single base pair resolution revealed that the globular domain of H1 (GH1) binds to the DNA minor groove located at the center of the nucleosome and contacts a 10-bp region of DNA localized symmetrically with respect to the nucleosomal dyad. In addition, GH1 interacts with about one helical turn of DNA in each linker region of the nucleosome and organizes thus path of the linker DNA at this region. Interestingly, a seven amino acid residue region (121–127) in the COOH terminus of histone H1.5 was required for "closing" of the linkers and the formation of the stem structure [46,47]. These data suggested that GH1 interact simultaneously with the dyad and with about one helical turn of the linker DNA to either side of the core region [46]. The recent electron cryo-