Histone H1, the forgotten histone

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First draft submitted: 17 January 2019; Accepted for publication: 28 January 2019; Published online: 22 February 2019

Keywords: chromatin • H1 • linker histones • nucleosome • posttranslational modifications

DNA is packed in nucleosomes, which form the building blocks of eukaryotic chromatin. The major protein component of chromatin are histones, which can be grouped into core and linker histones. In recent years, the research community focused mainly on the core histones and gained a broad understanding of their roles in gene regulation. In contrast to the core histones, the linker histone H1 is still understudied. For a long time, H1 has been considered as a rather unexciting general condenser of chromatin. However, more recently it has become clear that H1 has the potential to fine-tune transcription in a locus-specific manner rather than being a mere transcriptional repressor [1,2].

Two of each of the four core histones H2A, H2B, H3 and H4 form a histone octamer around which approximately 146 bp of DNA are wrapped [3]. The fifth histone H1 binds to these nucleosomal core particles close to the DNA entry and exit sites and protects the free linker DNA (~20 bp) between the individual nucleosomal core particles. These full nucleosomes (also referred to as chromatosomes) are necessary for the formation of higher order chromatin structures *in vivo* [4]. Although the structure of the nucleosomal core particle has been solved two decades ago, the structure of the full nucleosome and especially H1 structure and its precise localization remains a controversy in the field [3]. Two H1 binding modes have been proposed, first an asymmetric (off-dyad) H1 binding, where H1 binds to one of the two linker DNAs and the nucleosomal DNA adjacent to the dyad axis. Second a symmetric (on-dyad) binding, where H1 contacts both linker DNAs and the nucleosomal DNA close to the dyad axis [5]. In common for both modes is that H1 binding leads to a reduction in the angles of the DNAs entering and exiting and impacts on nucleosomal breathing. The precise binding of H1 might thus be more flexible than originally expected and might be governed by the individual H1 subtypes, its posttranslational modifications (PTMs) and/or the DNA sequence.

Linker histones are the most divergent histone protein family comprising 12 subtypes that can be expressed in human and mice. The individual subtypes can be grouped into three distinct groups: first the somatic replication-dependent subtypes (H1.1–H1.5) that are expressed mainly in the S-phase, second the somatic replication-independent variants (H1.0 and H1.10) expressed during the complete cell cycle and third the germ line-specific (testis or oocyte) subtypes (H1.6 (TS), H1.7 (TS), two splice variants of H1.8 (OO) and H1.9 (TS)). All H1 are highly lysine rich and share a similar tripartite structure containing an unstructured short N-terminus (~40 aa), a central globular domain (~70–80 aa) and a very long unstructured C-terminus (>100 aa) (Figure 1) [6]. Such unstructured or intrinsically disordered regions, as found in the H1 N and C-termini, are key factors driving membrane-less compartmentalization of nuclear clusters, often referred to as phase separation. Indeed it has recently been shown that the H1 C-terminus can undergo phase separation *in vitro* [7]. Whether this H1 droplet formation occurs *in vivo* and is subtype specific has not been studied yet.

Individual subtypes are more conserved between rather than intraspecies; for example, H1.1 in humans and mice are more similar than human H1.1 compared with human H1.2. This contributes to the concept that H1 heterogeneity might be evolutionarily conserved and that individual H1 subtypes have specific functions. In line with this, individual subtypes display differential chromatin-binding kinetics *in vivo*. H1.1 and H1.2 are the most mobile subtypes whereas H1.4 and H1.5 are the most immobile ones [11]. Also pointing toward specific functions are our results that the expression of the somatic replication-dependent H1 subtypes during early mouse development is dynamic and stage specific as well as that changes in H1 subtypes might be necessary for

Figure 1. Posttranslational modifications of murine H1 subtypes. Amino acid sequence alignment and domain organization [8,9] of murine somatic replication-dependent H1 subtypes. Consensus amino acid sequence is presented as a position weight matrix. H1 subtype-specific PTMs according to Starkova *et al.* 2017 [10] and phosphosite.org (only PTMs with at least five publications listed, note that mH1.1 is not included in this database).

epigenetic reprogramming [12]. Therefore in our opinion it is important to study individual H1 subtypes rather than conducting studies with ‘bulk H1’. One should note that for *in vitro* studies (including structures) often chicken H5 or *Drosophila* H1s have been used and these H1 variants differ in their biophysical properties from mammalian H1s, so conclusions are not necessarily transferable to human or mouse systems.

Studying PTMs of H1 is challenging mainly due to the high number of different modifications and high content of lysine residues that result in limited sequence coverage when using standard MS approaches. Nevertheless, it is evident that H1s are extensively posttranslationally modified like their core histone counterparts. For example, PTMs such as methylation, acetylation, ubiquitination, formylation, poly-ADP ribosylation and phosphorylation have been identified on all H1s in different organisms [6,13] (Figure 1). Cell cycle dependent C-terminal H1 phosphorylations have been most extensively studied and can be clearly linked to global chromatin de-/condensation [13]. Unraveling the functional relevance of nonphosphorylation H1 PTMs is currently in its infancy and these modifications need to be extensively studied in the future. However, some interesting examples have been characterized in the last few years. For example, specific H1 modifications such as H1.4K34 acetylation (in the N-terminus) can have multiple functions; they can recruit transcription factors as well as affect H1 mobility and hence regulate transcription [14]. The level of H1.4K34 acetylation has also been linked to stemness of cells and is altered in different types of cancers [14]. Interestingly histone modifiers can modify specific H1 subtypes at distinct positions; for example, G9a/KMT1C and Glp1/KMT1D can methylate H1.4 in the N-terminus at K26 and H1.2 in the C-terminus at K187 [15]. The existence of such H1 subtype-specific modifiers adds an exciting new layer of complexity to our understanding of H1 *in vivo*, but also causes an additional challenge since it necessitates subtype- and modification-specific antibodies.

The ratio of H1 to nucleosomal core particles is roughly between 0.5 and 0.8, depending on the cell type, and can impact the nucleosomal repeat length [16]. Initial studies on the genomic localization of bulk H1 revealed H1 depletion at transcription start sites, enhancers and CTCF binding elements. Subtype-specific chromatin

immunoprecipitation (ChIP) studies are currently limited by the absence of subtype-specific antibodies. Multiple studies therefore used tagged exogenously expressed H1s, which could result in compensatory changes in the H1 subtype composition. H1 subtype-specific DamID, which avoids this problem, revealed that somatic H1 subtypes can be divided into two groups based on their genomic distribution. H1.2 to H1.4 are depleted from active promoters and enhancers and H1.1 does not show such a clear profile and might contribute to a ground-state chromatin architecture compatible with active transcription [17,18]. To date no comprehensive ChIP-seq experiments covering all H1 subtypes and avoiding ectopic expression have been conducted in the same cellular background.

The distinct genomic localization and different modifications between the H1 subtypes might also suggest the existence of H1 subtype-specific interactors. However, a systematic proteomic analysis of the interactome of individual H1 subtypes including both chromatin bound and free is still missing. Previous studies often used recombinant proteins or focused on the H1.0 replacement variant. The described H1-protein interaction partners encompass pre-mRNA splicing factors, histone chaperones, components of the transcription machinery, DNA-damage response factors and even components of the translation machinery, which demonstrates that H1 is involved in diverse processes [19]. Specific interactors might recognize individual H1 subtypes due to the differences in their different N- and C-termini (Figure 1). For example, it is known that the DNA methyltransferases DNMT1 and DNMT3B can interact specifically with the H1.3 subtype [20].

In recent years, changes in H1 composition and expression levels have been found in several cancers and other diseases. A reduction of bulk H1 mRNA levels of up to 40% has been observed in ovarian adenocarcinoma cancer [21]. The expression levels of the individual subtypes are also altered in several malignancies; for example, H1.5 expression is increased in metastatic prostate cancer cells and several other cancers [22]. However, the functional consequences of altered H1 expression levels and changes in the H1 composition are only poorly understood. Additionally, point mutations of histone H1 subtypes have been found in different types of cancers. However, in contrast to the well-characterized oncomutations in the core histones (e.g., H3.3 K27 to M) H1 point mutations are currently not well studied.

In conclusion, H1 is a major chromatin component that is currently understudied mainly due to the complexity of the subtypes expressed and the lack of subtype-specific antibodies. Many studies have been conducted with either the H1.0 replacement variant, total H1 or only selected individual H1 subtypes from different organisms. Nevertheless the published results point toward an underestimated functional heterogeneity of H1. In our opinion future H1 studies should be conducted in a systematic way including all H1 subtypes, such as for ChIP-seq where all H1 subtypes present are mapped in the same cell. Although the H1 modification pattern seems as complex as core histone modification, little is known about the function of H1 modifications. Subtype-specific H1 PTMs might determine the H1 binding dynamics and thus chromatin accessibility, the genomic location of H1 subtypes and/or regulate individual H1 interactomes. Furthermore, manipulation of histone modification levels, by ‘epidrugs’ such as HDAC inhibitors also affect H1 modifications and hence H1 dynamics – a fact often ignored in the field. At the end it should be noted that we are just starting the journey of unraveling H1 subtype-specific functions in the chromatin context and we highly encourage scientists to further investigate this interesting histone class.

Acknowledgements

We apologize to the many colleagues whose work was not cited due to space limitations. We thank P Bheda for helpful discussions and proofreading.

Financial disclosure

Work in the RS Laboratory is supported by the DFG through SFB 1064 and SFB 1309, the EpiTrio consortium as well as AmPro program and the Helmholtz Gesellschaft. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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