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Linker histones in hormonal gene regulation☆

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ABSTRACT

In the present review, we summarize advances in our knowledge on the role of the histone H1 family of proteins in breast cancer cells, focusing on their response to progestins. Histone H1 plays a dual role in gene regulation by hormones, both as a structural component of chromatin and as a dynamic modulator of transcription. It contributes to hormonal regulation of the MMTV promoter by stabilizing a homogeneous nucleosome positioning, which reduces basal transcription whereas at the same time promoting progesterone receptor binding and nucleosome remodeling. These combined effects enhance hormone dependent gene transcription, which eventually requires H1 phosphorylation and displacement. Various isoforms of histone H1 have specific functions in differentiated breast cancer cells and compact nucleosomal arrays to different extents *in vitro*. Genome-wide studies show that histone H1 has a key role in chromatin dynamics of hormone regulated genes. A complex sequence of enzymatic events, including phosphorylation by CDK2, PARylation by PARP1 and the ATP-dependent activity of NURF, are required for H1 displacement and gene de-repression, as a prerequisite for further nucleosome remodeling. Similarly, during hormone-dependent gene repression a dedicated enzymatic mechanism controls H1 deposition at promoters by a complex containing HP1γ, LSD1 and BRG1, the ATPase of the BAF complex. Thus, a broader vision of the histone code should include histone H1, as the linker histone variants actively participate in the regulation of the chromatin structure. How modifications of the core histones tails affect H1 modifications and *vice versa* is one of the many questions that remains to be addressed to provide a more comprehensive view of the histone cross-talk mechanisms. This article is part of a Special Issue entitled: Histone H1, edited by Dr. Albert Jordan.

1. Introduction

The fundamental unit of chromatin is the nucleosome core particle (NCP), which consists of 147 bp of DNA wrapped in a left-handed superhelix around an octamer of core histones [1,2]. Members of the family of H1 histones or linker histones limit the mobility of NCPs by binding to it from outside with its central globular domain interacting with the nucleosome DNA at the pseudo-dyad axis and at the nucleosome entry and exit sites. The N- and C-terminal extensions of H1 histones contact the linker DNA that connects NCPs leading to compaction of the chromatin fiber. In chromatin digestion experiments with micrococcal nuclease (MNase) that cleaves preferentially linker DNA, chromatin free of linker histones generates a nucleosomal repeat length (NRL) of 145–150 nt, whereas H1 containing chromatin yields NRL of 170–190, depending on the H1 density. The particles containing NCP and linker DNA with associated linker histones are the nucleosomes, although frequently this term is used for NCPs and the complex with linker histones is called the chromatosome.

Within the cell nucleus, the nucleosomes fold to form a chromatin fiber of an approximate diameter of 30 nm, the precise structure of which is still a matter of debate. While the original description of the 30 nm fiber proposed a solenoid structure with 6 nucleosomes per turn, more recent results from cryo-EM imagines and X-ray crystallography favor and intertwined zig-zag structure with two nucleosome stacks forming a left turning helix [3]. *In vitro*, under appropriate salt conditions NCPs deposited on long DNA molecules form a “beads on string” structure with a diameter of 11 nm, which in the presence of linker histones condense into a 30 nm fiber. However, results obtained using small-angle X-ray scattering (SAXS), cryo-EM and more recently, stochastic optical reconstruction microscopy (STORM) have failed to detect the 30 nm fiber [4–6]. These latter studies support the hypothesis that nucleosomes exist mainly within the 10 nm fiber in eukaryotic nuclei with the possible presence of larger heterogeneous “clutches” of nucleosomes of varying size [6], in agreement with recent results using high resolution 3C-derived method (Micro-C) in yeast [7].

Although both the existence and the structure of the 30 nm fiber are still up for debate one thing is clear, the presence of H1 is essential for the higher order folding of chromatin. Histone H1 has been traditionally associated with chromatin compaction that limits nucleosome mobility and access of transcription factors or RNA polymerase and, as a

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consequence, favors transcriptional repression. This vision is in agreement with the observation that the stable condensed and silent heterochromatin in eukaryotic cells is enriched in linker histones, compared to the more open and active euchromatin. However, recent results suggest that linker histones may play a more plastic and complex role in the regulation of gene expression in the context of dynamic chromatin, which changes its protein composition in response to internal cell programs or to external cues.

2. Histone H1 Family

In mammals the histone H1 family consists of 11 closely related, single-gene encoded proteins, including seven somatic subtypes (from H1.1 to H1.5, H1x and the terminally differentiated isoform H1.0) and a few germ line specific subtypes (H1t, H1T2, HILS1, H1oo). As knockdown of individual somatic H1 subtypes in mouse has no marked phenotype [8], H1 variants have been assumed to be highly redundant. However, we found that inducible knockdown of individual somatic H1 subtypes in breast cancer cells altered the expression of different subset of genes, the majority of which were down-regulated upon H1 depletion [9]. This argues against a general repressive role of linker histones and suggests some non-redundant effects of the individual variants on gene expression. Indeed, depletion of individual subtypes had different effects on cell survival. Notably, H1.2 depletion specifically causes cell cycle arrest due to repression of key cell cycle genes, whereas H1.4 depleted cells eventually die of necrosis [9]. Moreover, although H1.2 accounts for approximately only 20% of the total H1 content in T47D breast cancer cells, its depletion caused a general decrease in nucleosome spacing, which is not compensated by the overexpression of other subtypes [9]. This suggests that individual somatic H1 subtypes have a selective effect on chromatin structure, supporting the idea that distinct roles do exist for the linker histone variants in differentiated cells.

Various living cell approaches including fluorescence recovery after photobleaching (FRAP), revealed dramatic differences between the individual histone H1 subtypes in terms of their chromatin binding affinity and their preference for euchromatin versus heterochromatin [10–12]. Using Atomic Force Microscope and sedimentation experiments we confirmed that H1 subtypes exhibit different affinities for minichromosomes assembled *in vitro* and different abilities to promote their condensation [13]. According to these experiments, H1 subtypes can be classified as weak condensers (H1.1 and H1.2), intermediate condensers (H1.3), and strong condensers (H1.0, H1.4, H1.5, and H1x). The variable C-terminal domain is required for nucleosome spacing by H1.4 and is likely responsible for the distinct chromatin condensation properties of the various subtypes, as shown using chimeras between *H1.4* and *H1.2* [13]. Moreover, using restriction enzyme accessibility we showed that linker histones do not preclude ATP-dependent remodeling of *in vitro* assembled minichromosomes by yeast SWI/SNF or *Drosophila* NURF [13]. Thus, linker histone subtypes can be considered as differential organizers of chromatin, rather than general repressors [13].

Genome-wide analysis of H1 isoform distribution in human lung fibroblasts has provided additional insights into the non-redundant functions of H1. Izzo and colleagues demonstrated that H1.2 to H1.5 are bound to repressive regions of the genome, while H1.1 is enriched at active regulatory regions, suggesting a unique function [14]. The chromatin structure promoted by H1.1 binding might support a level of compaction that facilitates rapid conversion into either an active or repressed state. Other genome-wide studies have confirmed specific behavior of particular variants. For instance, H1.0 is preferentially localized in nucleolus-associated DNA repeats, while H1.X exhibits a preferential association with RNA-polymerase II [15]. In T47D breast cancer cells recombinant HA-tagged H1 isoforms bind to chromatin in a relatively redundant manner as demonstrated by genome-wide correlation analysis. However, in isoform specific peak finding analysis, H1.2 and H1.3 exhibited some region specificity and H1.5 increased its chromatin binding after hormone induction (Pohl et al. unpublished). This is consistent with previous work performed in differentiated cells, which showed that knockdown of H1.5 resulted in aberrant gene expression and altered chromatin accessibility [16]. Although the evidence is mounting for a more specific role of each of the variants, the intriguing question still remains, as to what is the prevailing mechanism underlying preferential binding of particular H1 isoforms.

3. Role of histone H1 in gene regulation

In this review we summarize the advances in our knowledge on the role of histone H1 in T47D breast cancer cells exposed to nanomolar concentrations of R5020, a synthetic progestin (PG) acting via the progesterone receptor (PR). These cells respond to PG with a broad spectrum of changes including the activation of several signaling cascades that impact on chromatin [17]. In combination with various co-regulators tethered by the hormone receptors the action of the signaling pathways results in the transcriptional activation or repression of specific sets of genes [17–19]. The changes in gene expression require extensive changes in chromatin structure, a process in which H1 plays a key role [20]. More specifically, gene regulation results from a rapid activation of the SRC/ERK protein kinases signaling pathway by a fraction of PR anchored via palmitoylation to the cell membrane [21], followed by ERK mediated phosphorylation of nuclear PR and MSK1 [18]. This leads to the formation of an activated ternary complex PR-ERK-MSK1, which interacts with Hormone Responsive Elements (HREs) and initiates chromatin remodeling as required for transcriptional regulation [22].

3.1. Studies on the MMTV promoter

Initial insight into the role of nucleosomes in gene regulation by PR was obtained in *in vitro* studies with minichromosomes assembled from circular plasmids in extracts of preblastodermic *Drosophila* embryos. These extracts contain an excess of core histones but are deficient of linker histones, provided the eggs have not reached mid-blastula transition. Assembling the Mouse Mammary Tumor Virus (MMTV) promoter into minichromosomes in this system and using HeLa cells as a source of the transcription machinery we reproduced the physiological synergism between PR and Nuclear Factor 1 (NF1), which is a hallmark of hormonal induction that is not observed with naked DNA templates [23]. Moreover, whereas PR and NF1 compete for binding to their respective sites on the MMTV promoter when tested on free DNA [24], they bind synergistically to the same DNA assembled in nucleosome arrays [23], thus mimicking what was found in living cells [25].

It had been reported, that the MMTV promoter chromatin is depleted of histone H1 after hormonal induction [26], but overexpression of histone H1 in cultured cells enhances hormonal *trans*-activation of the promoter [27]. Our first observation indicating that linker histones may not simply act as repressors of transcription, emerged from experiments using in vitro assembled MMTV minichromosomes. First, we found that when added at stoichiometric amounts (1 per nucleosome) H1 bound asymmetrically to the MMTV nucleosomes and improved the translational positioning of nucleosomes over the MMTV promoter. This resulted in a decrease of both basal transcription, as well as transcription in the presence of either PR or NF1 [28]. Unexpectedly however, in the simultaneous presence of PR and NF1 the transcriptional synergism between the two factors was enhanced [28]. We concluded that H1 plays an important role during the *in vitro* activation of the MMTV promoter by favoring a dominant nucleosome positioning that precludes access of the basal transcriptional machinery and of NF1 making the promoter exquisitely dependent on PR. In the same line, using as a system the MMTV promoter reconstituted in *Xenopus oocytes*, which are deficient in somatic histone H1, the group of Orjan Wrange described that subsaturating expression of histone H1 enhanced

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