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Molecular dynamics of histone H1☆

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ABSTRACT

The H1 or linker histones bind dynamically to chromatin in living cells *via* a process that involves transient association with the nucleosome near the DNA entry/exit site followed by dissociation, translocation to a new location, and rebinding. The mean residency time of H1 on any given nucleosome is about a minute, which is much shorter than that of most core histones but considerably longer than that of most other chromatin-binding proteins, including transcription factors. Here we review recent advances in understanding the kinetic pathway of H1 binding and how it relates to linker histone structure and function. We also describe potential mechanisms by which the dynamic binding of H1 might contribute directly to the regulation of gene expression and discuss several situations for which there is experimental evidence to support these mechanisms. Finally, we review the evidence for the participation of linker histone chaperones in mediating H1 exchange. This article is part of a Special Issue entitled: Histone H1, edited by Dr. Albert Jordan.

1. Introduction

In late 2000 two papers describing the binding properties of linker histones to chromatin in living cells were published simultaneously in Nature [1,2] Both reports, one using mouse cells [1] and the other a human cell line [2], presented evidence that most of the H1 molecules were rapidly exchanging between chromosomal locations. Both studies used photobleaching techniques, primarily fluorescent recovery after photobleaching (FRAP), to demonstrate that at any given moment the vast majority of H1 molecules are bound to chromatin, with individual molecules residing at one location for only a minute or two before dissociating and rapidly translocating and binding to another random site. Notably, this exchange process appears to be ATP-independent and does not require fiber–fiber interactions [2]. These observations were somewhat surprising, because the prevailing view at the time was that chromatin was a relatively static macromolecular structure designed to package DNA into the confines of the nucleus [3,4]. While it was appreciated that DNA in the context of chromatin had to be at least transiently accessible to molecules that mediate processes such as replication, repair, and transcription, it was thought that this access was mediated primarily by active ATP-dependent complexes [5]. Linker histones in particular were considered to promote, or at least stabilize, the condensation of chromatin into the 30 nm fiber, the first level of higher order chromatin structure [6–10]. Thus, the results from these studies [1,2] suggested a new view, that bulk chromatin was inherently fluid, perhaps transitioning regularly from a condensed to a more open configuration. In retrospect, previous studies suggested that linker histones have the capacity to exchange between DNA molecules [11,12], but the idea that the bulk of the population in living cells was continuously exchanging on the time scale of minutes was unexpected.

In vitro studies had shown that in addition to stabilizing nucleosomes, H1 binding inhibits nucleosome sliding [13,14] and the transient exposure of DNA on the nucleosome surface [15,16]. Incorporation of H1 into *in vitro* assembled chromatin templates reduces subsequent transcription and inhibits remodeling by ATP-dependent complexes [17,18]. A prevailing view at the time was that chromatin functioned as a general repressor of transcription and the linker histones served as "gatekeepers" to regulate access to the underlying DNA [6,19–21]. Displacement of H1 was considered to be limited to sites within the genome that were undergoing processes that required chromatin decondensation and the local recruitment of factors to mediate displacement [20,22]. The observation that the bulk of the linker histone population was inherently in flux lead to an alternative view [23–30]. In this scenario at any given moment the linker DNA of most nucleosomes would be bound by H1. However, for any given nucleosome the H1 would dissociate every minute or so, leaving the linker DNA transiently accessible to other factors. These factors might be a number of transcriptional factors, chromatin architectural proteins, or histone/chromatin modifying enzymes with demonstrated affinity for nucleosomal linker DNA [31]. The transient or stable replacement of H1 with these factors or the action of these factors on nucleosomal DNA or core histones could promote the formation of a chromatin environment conducive to a particular process, for example transcription or repair. Many of these factors are known to roam the nucleus and interact

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transiently with DNA [27], and there is considerable evidence that a network of nucleosomal-binding proteins operates within this scenario to modulate chromatin-mediated processes. Thus, in a loose sense, the dynamic binding of linker histones might be considered part of a mechanism for regulating these processes. However, an important consideration is whether H1 dynamics plays an active or passive role. One possibility is that the propensity for H1 to exchange positions is an inherent biophysical property that has been evolutionarily selected to allow for the scenario described above. In this sense the outcome from binding of non-H1 proteins would be driven mainly by the local concentration of these factors and their affinity for nucleosomal DNA relative to that of the linker histones. A more intuitive and inherently attractive possibility is that the dynamic binding properties of linker histones can be modified in response to physiological clues to favor a particular outcome. However, definitive demonstration that a particular alteration in the linker histone's interaction with chromatin functions directly to mediate a particular physiological outcome requires a more critical evaluation.

In the 15 years since the initial demonstration that linker histones interact dynamically with a chromatin [1,2], a great deal has been learned about the exchange process and factors that might influence it. Additionally, the development of techniques such as photobleaching and related methodologies has facilitated studies towards understanding additional aspects of chromatin biology. These results will be discussed in this review with an eye towards critically evaluating the evidence for regulation of H1 dynamics to mediate specific physiological outcomes. Due to space restrictions this review will focus mainly on mammalian systems using the proposed unified nomenclature [32].

2. The kinetic properties of H1 binding

Most of the information related to H1 binding *in vivo* has been generated with the technique of fluorescence recovery after photobleaching (FRAP). The principle of an H1 FRAP experiment is fairly straightforward [33–35]. A small subregion of the nucleus of a cell expressing a fusion of an H1 protein with a fluorescent tag, usually green fluorescent protein (GFP) or a related protein, is irreversibly "bleached" or driven into a dark state by a brief pulse of high intensity laser light. The rate at which fluorescence returns or recovers within the bleached region is then monitored and quantified. Recovery involves the movement of bleached molecules out of the bleached region and their replacement by unbleached molecules from elsewhere in the nucleus. The rate at which this occurs reflects the mobility of the protein and provides information on the diffusion rate and any binding events that occur between the protein of interest and immobile binding sites throughout the nucleus. For the H1 proteins, these binding sites are nucleosomes. It should be appreciated that FRAP experiments must be interpreted properly. These issues have been addressed in a number of reviews [33,36–38] and will not be discussed in detail here. Much of the challenge in interpreting FRAP recovery data is to distinguish between the contributions of binding and diffusion rates, if these values are similar. For functional wild type linker histone-GFP constructs, the observed recovery times, generally on the order of minutes, are much longer than that of diffusion-limited processes. Furthermore, because there is an insignificant amount of unbound H1 at any time, the recovery curve is considered to be diffusion-uncoupled and the measured recovery time is a direct measure of the affinity of the H1 for the chromatin binding site. Most primary data is reported as t_{50} values, the time to recover to one half the pre-bleach value, corrected for loss of fluorescence during imaging, or to a plateau. In both of the original FRAP papers [1,2], fluorescence recovery into the bleached region reached a plateau that was significantly less than the pre-bleach intensity. This was interpreted as evidence for two kinetic classes; one mobile and the other immobile. In mouse cells the mobile fraction comprised 75% and 95% of the total in heterochromatic and euchromatic regions, respectively [1]. However, the immobile class was deemed so only because it did not recover within the time frame of the experiment. A more recent, extremely thorough analysis of the kinetic behavior of human H1 subtypes revealed the existence of at least two major mobile classes of kinetic binding behavior [39]. One class, referred to as low affinity, displayed residence times of approximately 30–100 s depending on the H1 variant. In contrast, the high affinity class displayed residence times of approximately 2.5–12 min. It should be noted that about 80–85% of each variant displayed low affinity binding, and that this binding is not representative of a significant freely diffusing population. This suggests that individual molecules can transition from low affinity to high affinity binding. The structural differences between the binding classes are unclear but it is likely that any of a number of post-translational modifications of H1 may contribute to this transition [40–45].

In addition to FRAP, a full and accurate picture of the binding properties of any individual chromatin-binding protein will require the systematic application of multiple techniques such as fluorescence correlation spectroscopy (FCS), raster image correlation spectroscopy (RICS), single molecule tracking (SMT) and competition ChIP [46–51]. These techniques all have specific advantages and drawbacks, but their simultaneous application will allow cross validation and provide a more accurate determination of the processes occurring in living cells. Nevertheless, information derived primarily from FRAP analyses can be extremely informative. For perspective, FRAP analysis of core histones revealed residence times of several hours [52] consistent with the notion that most intact nucleosomes, at least those not located in chromatin undergoing active transcription or replication, are relatively static. In contrast, the residency time of chromatin-binding proteins such as the high mobility group (HMG) proteins, heterochromatin binding protein one (HP1), and most transcription factors are much faster, typically in the range of a few seconds [27,28,53]. In practice, the binding behavior of the linker histones, with residency times of minutes, makes them particularly amenable to FRAP-based experimental approaches to elucidate the molecular details of the binding process.

3. From kinetics to structure and back again

In mammalian species the H1 histones exist as a family of ten or more non-allelic primary sequence variants, or subtypes [54–58].The major somatic variants have a conserved tripartite structure composed of a conserved central globular domain consisting of a three helix bundle containing a winged-helix fold, flanked by a long, extremely lysine rich C-terminal tail and a short, flexible N-terminal tail enriched in basic amino acids and proline [4,59]. Mutations in each of these domains have been shown to influence exchange kinetics, indicating that all three domains contribute, to some degree, to the binding affinity of the intact H1 molecule to chromatin [60–62].

3.1. H1 dynamics provide structural information

Photobleaching experiments have also been utilized to obtain structural information pertinent to the location of H1 within the nucleosome. Based primarily on *in vitro* experiments, the central globular domain of H1 was believed to bind near the nucleosome dyad and to make contact with two or three strands of DNA [9,63–66]. The crystal structure of the globular domain of avian H5 (GH5), which is 97% homologous to that of mouse H1.0 had been solved in the absence of DNA [67]. As linker histone binding shows little sequence specificity and is largely electrostatic in nature [68], it is likely that binding of the globular domain is driven primarily by interactions between DNA and positively charged amino acid residues on the surface of GH5. Inspection of the structure revealed two well separated clusters of basic amino acids. Simultaneous mutation of all the residues within these sites abolished the ability of the purified GH5 to bind to H1-depleted oligonucleosomes [64,66] However, individual single mutations of residues within these clusters did not affect DNA-binding, somewhat limiting the interpretation. Fortunately, FRAP analysis proved to be sensitive enough to measure the contribution of individual amino acids [69]. A set of H1.0-GFP fusions, in which

each of the 16 basic amino acids on the surface of the globular domain of mouse H1.0 were individually mutated to neutral residues were expressed in mouse fibroblasts and analyzed by FRAP. Remarkably, the mutants fell into two distinct classes. Mutations of nine residues resulted in major reductions in the FRAP recovery time, indicating that these residues make critical contributions to H1.0 binding *in vivo*. The other seven mutants had little or no effect on FRAP recovery. Mapping of the binding residues onto the crystal structure of the GH5 revealed that they were located in two distinct clusters, termed site 1 (S1) and site 2 (S2), on one face of the molecule (Fig. 1). All of the non-binding residues were distributed on the opposite face. Molecular modeling was then used to propose a model for the binding geometry of the H1.0 globular domain within the nucleosome. In this asymmetric model, one binding site (S1) interacts with the major groove near the nucleosome dyad and the other (S2) with one strand of linker DNA. Since presentation of this model other studies and models have appeared, some of which are consistent with this asymmetric model while others support a symmetrical model with the globular domain binding at the dyad and contacting both entering and exiting linker strands [70–74].

While this manuscript was under revision, Zhou et al. [75] presented a crystal structure of GH5 bound to a positioned nucleosome. The derived structure is remarkably similar, albeit with some minor details, to that predicted from the FRAP studies. The crystal structure indicates that GH5 binds symmetrically at the nucleosome dyad and interacts with both the entering and exiting DNA linker strands. All of the binding residues identified by FRAP are involved in binding interactions with nucleosomal DNA in the crystal structure. Two residues in site S1, His-25 and Arg-47, predicted to bind near the dyad in the model originally presented [69], interact with linker DNA in the crystal structure. That a similar structure was derived from two very different experimental approaches, each with its own set of limitations and caveats, is encouraging.

The globular domain of the H1.0 subtype displays significant sequence divergence from that of the other somatic variants, which are highly conserved among themselves [55–57]. A high resolution NMR structure of the globular domain of chicken ortholog of mouse H1.2 predicted an overall structure nearly identical to that of GH5 [76]. The mutagenesis/FRAP approach described above was applied to the globular domain mouse H1.2 subtype to identify putative binding sites [77]. While binding residues could easily be distinguished from nonbinding residues, when these where mapped onto the NMR structure to generate a map of the interaction surface of H1.2, the result was distinct from that of H1.0 (Fig. 1). Furthermore, attempts to model the H1.2 globular domain into the site determined for that of H1.0 failed, indicating that these two linker histone subtypes interact with nucleosomes with very different geometries. Interestingly, based on their structural studies and observations on modeling studies with Drosophila H1, Zhou et al. [75] reached a similar conclusion. The structure of the 30 nm fiber has been hotly debated for decades, but the growing opinion is that *in vivo* chromatin is highly polymorphic [78,79]. An intriguing speculation is that different H1 subtypes might be more easily accommodated into or even preferentially stabilize different forms of the 30 nm fiber.

3.2. A kinetic model for H1 binding

Through the use of domain swap constructs, deletions and point mutations of human subtypes, it was concluded that the C-terminal domain is the primary determinant of H1 binding [60]. Notably, deletion of the C-terminal domain or a specific point mutation within that domain completely abrogated binding [61]. Subsequent studies using the mouse H1.0 subtype showed that any number of point mutations within the globular domain severely compromises nucleosomal binding *in vivo* [69], arguing that the globular domain is the primary determinant of binding. These apparently contradictory results can be reconciled if the two domains cooperate in the binding process; that is, binding of one facilitates binding of the other. The quantitative data generated in the H1.0 modeling paper precipitated considerations regarding the kinetic pathway of the binding reaction. Constructs containing multiple mutations in either of the clusters of binding residues within the globular domain (S1 or S2) recovered in ~1 s, essentially a completely null or nonbinding mutant. Furthermore, single mutations in any key binding residues resulted in very short recovery times approaching those expected for a completely non-binding protein. This behavior suggests that these sites and residues are acting synergistically or cooperatively to promote binding. Based on these observations, a model for the kinetic pathway was proposed [69]. In this model the C terminus binds efficiently but nonspecifically to linker DNA, bringing the globular domain into the vicinity of the nucleosome to facilitate specific binding, which in turn promotes the acquisition of specific structures within the subdomains of the C terminus to facilitate chromatin condensation [80,81]. This model was corroborated and refined by Stasevich et al. [82] using a set of constructs bearing null mutations in either of the globular domain sites or both in the presence or absence of the C terminus, and a modified FRAP procedure was used to allow measurements of cooperativity. The data indicate two possible pathways, both initiated by binding of the C terminus, which enhances the binding of either S1 or S2 with a cooperativity factor of 2.5. Upon binding of two sites, that is the C terminus and either S1 or S2, binding of the third site is enhanced by a cooperativity factor of ~25. In this model the acquisition of secondary structure upon binding of the C terminus results in a conformational change that promotes efficient and cooperative binding of the globular domain. This model is particularly attractive because the pathway evokes the presence of several partially bound intermediate states. As previously described, a proposed mechanism for the modulation of

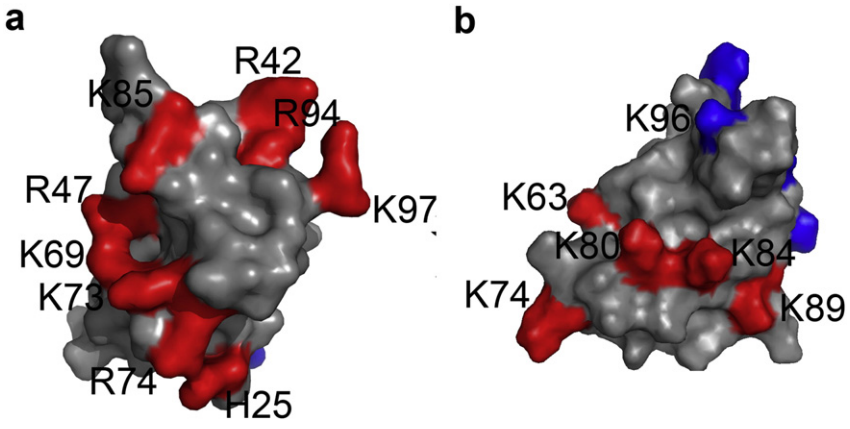

Fig. 1. Maps of the interaction surfaces of the globular domains of the mouse H1.0 (a) and H1.2 (b) linker histone variants. Red, binding residues; blue, nonbinding residues.

chromatin structure to regulate transcription involves a network of nucleosomal binding proteins that compete for binding with H1. These competing molecules might target these metastable bound states, which might be present for some fraction of the "residence time" of H1 determined from FRAP experiments of intact wild type linker histones.

4. The role of linker histone dynamics in cellular regulation

Most somatic tissues express six major subtypes, H1.0, H1.1–H1.5, and there is considerable evidence that there is a functional significance to this heterogeneity [54–56] although the molecular mechanisms are not well understood. With respect to H1 dynamics, two major questions often arise; are there differences among the subtypes in their quantitative chromatin-binding properties and do these differences have functional significance?

The answer to the first question is clearly yes. The Hendzel lab conducted a thorough FRAP analysis of all six major somatic H1 subtypes and demonstrated that there are significant quantitative differences in the low affinity and high affinity binding properties among these proteins [39]. Analysis of mouse variants is not as complete, but clear differences among variants have been identified [43,62,77]. Furthermore, somatic histones are reversibly phosphorylated in interphase and mitosis, often in a subtype-specific pattern, and this modification alters the affinity of H1 for nucleosomes [40–43]. Additional posttranslational modifications of H1 have been recently described and evidence has been presented that these modifications influence the dynamic interaction of H1 with chromatin [44,45]. This topic will not be discussed in detail here as it is covered in several reviews in this series.

The answer to the second question, regarding the functional significance of differences in dynamic binding properties among variants is also probably yes, although definitive evidence is lacking. For example, although depletion or over-expression of specific H1 variants has been shown to have phenotypic consequences on gene expression and/or cell cycle progression [83–87] these effects have not been ascribed specifically to the binding properties of the mis-regulated variant.

As previously described the observation that in addition to H1, other chromatin-binding proteins exhibit dynamic binding properties led to the proposal that a network of interacting or competing binding events might guide transitions in chromatin structure. In essence, the transient dissociation of H1 provides a window of opportunity for other factors to bind and, perhaps through subsequent alterations to chromatin structure (*i.e.* addition of posttranslational modifications or recruitment of remodeling factors), create a stable or metastable structure that persists long enough to allow a process such as transcription to occur. This scenario would require that such factors be present in high enough local concentrations and possess enough affinity to remain bound long enough to accomplish this before another molecule of H1 could appear to displace it. It is not clear to what degree this might actually occur *in vivo*, therefore we will discuss three factors for which substantial evidence does exist.

4.1. Pioneer factors mimic H1

Proteins such as HNF3 (FoxA) are termed pioneer transcription factors as they are believed to act early in development to alter "naïve" chromatin to enable subsequent gene activation [88]. FoxA, which is required for the initiation of liver development [89], and other members of this family have a tripartite structure that is very similar to H1 (Fig. 2). The central "winged helix" DNA-binding domain is remarkably similar in structure to the central globular domain of H1, suggesting that they are derived from a common ancestor [67,90]. Results from *in vitro* assays of binding to compacted nucleosomal arrays as well as *in vivo* FRAP analysis of mutant and wild type constructs lead to the following conclusions [91–93]. The globular domain of FoxA has non-specific sequence affinity for nucleosomal DNA that approaches that of linker histones, but can also bind with high affinity to specific consensus sequences upstream from target genes. The C terminus of FoxA, in contrast to that of the linker histones, interacts with core histones rather than linker DNA and promotes an open chromatin conformation rather than chromatin compaction. The following picture of FoxA function thus emerges. FoxA can slowly and deliberately scan the genome by interacting with nucleosomal DNA as it becomes accessible following H1 dissociation. Most of these interactions would be transient, as the residence time of FoxA binding to bulk chromatin is about half that of H1. However, if FoxA encounters its specific binding motif, this would result in much tighter binding and retention on the nucleosome. In this state, interactions of the C terminus with core histones could promote an open chromatin conformation allowing additional factors to bind to establish an active transcriptional complex or the establishment of epigenetic marks to promote subsequent expression.

Other pioneer factors, such as those involved in reprogramming somatic cells to pluripotency (Oct4, Sox2, Klf4) do not structurally resemble linker histones. These factors are able to recognize partial DNA motifs on nucleosomes and, often acting in concert, promote establishment of an open chromatin structure conducive to reprogramming [94]. It is conceivable that these partial motifs, because of their smaller size, might become accessible when H1 is bound in a metastable state as part of the kinetic pathway for binding (see Section 3.2), Interestingly, a recent report demonstrated that linker histones undergo site-specific citrullination in pluripotent cells and that this modification results in displacement of H1 from nucleosomes and global chromatin decondensation [45].

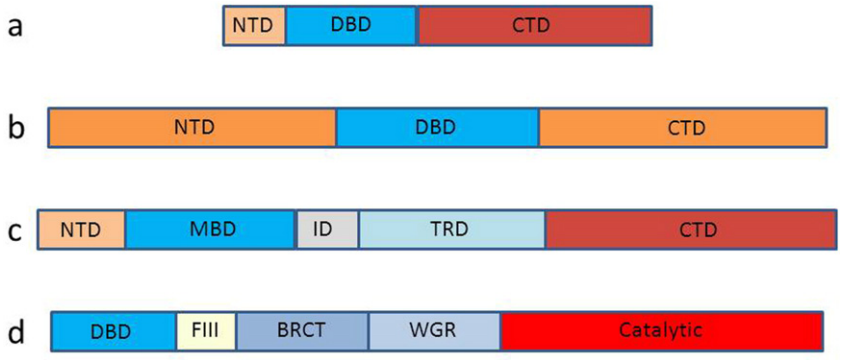

Fig. 2. Domain structure of nucleosome binding proteins. a) linker histones (~220 aa); b) FoxA (468 aa); c) MeCP2 (486 aa); d) PARP-1(1014 aa). Abbreviations: NTD, N-terminal domain; DBD, DNA-binding domain; CTD, C-terminal domain; MBD, methylated DNA binding domain; ID, intrinsically disordered domain, TRD, transcriptional repression domain; FIII, zinc finger motif; BRCT, BRCA1 C-terminus domain; WGR, Trp, Gly, Arg domain.

4.2. Neurons use an "alternative" linker histone

Methylated CpG Binding Protein 2 (MeCP2) is a nuclear protein which selectively recognizes methylated DNA and was thought primarily to recruit histone deacetylases to establish a repressive chromatin structure at specific chromatin loci such as gene promoters [95]. This protein is of particular clinical interest as mutations in the X-linked *MECP2* gene are responsible for the autism spectrum disorder Rett syndrome [96]. It is now recognized that MeCP2 can also bind to unmethylated DNA in the context of chromatin and may be involved a number of chromatin-related activities in addition to gene repression [97]. Importantly for this discussion, MeCP2 can compete with linker histones for nucleosomal binding sites to potentially influence chromatin higher order structure [98,99]. The structure of the MeCP2 protein is inherently interesting (Fig. 2). There are multiple domains within the protein that apparently interact to mediate DNA binding *in vivo* [97]. The MBD domain is responsible for not only mediating selective binding to methylated DNA, but can also bind tightly to unmethylated DNA. Several other domains, including NTD, ID, TRD and CTD, possess non-specific DNA binding properties or can influence binding by the MBD domain. Additionally, mutations that are associated with Rett syndrome are found in each of these domains. A recent report provides insight into the specific dynamic interactions between binding of H1 and MeCP2 to nucleosomes. Using *in vitro* assembled nucleosomal substrates, Ghosh *et al.* [99] demonstrated that MeCP2 binds cooperatively to nucleosomes with a stoichiometry of 2:1 to generate an architectural motif near the entering and exiting linker DNA strands that approximates the stem motif identified for linker histone binding [10]. Using methylated mononucleosomal substrates with linker DNA extensions, they further demonstrated that binding of MeCP2 and H1 were mutually exclusive and MeCP2 was capable of displacing H1 more effectively than *vice versa*. Next they used an approach involving microinjection and FRAP to explore the relationship between MeCP2 and H1 binding *in vivo*. FRAP analysis of MeCP2 revealed the presence of both a mobile and an immobile fraction, indicating that MeCP2, like H1, exhibits both low affinity and high affinity binding. Evidence was presented to indicate that high affinity binding represents MeCP2 binding to nucleosomes containing methylated DNA. When cells stably expressing H1.0-GFP were microinjected with MeCP2, there was a significant increase in overall mobility and a small increase in the amount of H1.0-GFP in the immobile fraction. In the converse experiment, in which cells expressing MeCP2-GFP were challenged with microinjected H1.0, there was also an increase in the rate of exchange within the mobile fraction but also a striking increase in the amount of stably bound MeCP2. This was interpreted to indicate that while H1.0 and MeCP2 compete effectively for "low affinity" binding (the mobile fraction), H1.0 is much less effective in competing with MeCP2 binding for high affinity sites, presumably those involving nucleosomes containing methylated DNA, consistent with the results from the mononucleosome studies. As the authors point out, these observations imply that considerations of the significance of chromatin binding by nuclear proteins must account for the relative abundance of potentially competing components and their affinities for specific and/or nonspecific targets [99]. Hansen *et al.* [97] further note that in cells in which the level of MeCP2 is much lower than that of H1, MeCP2 is be predicted to be involved primarily in local, gene specific functions. In contrast, if levels of MeCP2 were much higher MeCP2 might also be involved in global chromatin modulation. A recent report demonstrating that the levels of MeCP2 in neurons are extremely high, approaching histone octamer levels, provides interesting evidence that this is exactly what occurs [100]. In neurons, the amount of H1 is approximately half that found in most other cells. As MeCP2 binds to nucleosomes as a dimer, and since a single nucleosome cannot bind MeCP2 and H1 simultaneously, the results indicate that in neurons about half the nucleosomes are bound to H1 and half to MeCP2. Considering the numbers of CpG sites, it was concluded that there are enough MeCP2-containing nucleosomes to "saturate" all the CpG sites. High throughput sequencing revealed that MeCP2 does track methyl-CpG density in neurons. In the *Mecp2* null brain, the levels of H1 are twice that of wild type neurons, suggesting that MeCP2 might function as an "alternative" linker histone in normal neurons. This is certainly plausible based on the aforementioned data that MeCP2, like H1, binds to linker DNA and promotes the formation of higher order chromatin structures. This begs the question why MeCP2 is replacing H1 specifically in neurons. The authors present two possible explanations. One is that neurons are inherently sensitive to transcriptional "noise" from repetitive elements and that MeCP2 is acting as a global repressor to suppress this by recruiting HDAC activity. Another proposal is that neuronal plasticity and homeostasis demand a rapid dynamic response in gene expression patterns, for example upon synaptic firing. In this view MeCP2, *via* any of its multiple domains that influence chromatin binding, might be, relative to H1, considerably more adept at responding to physiological cues. In support of this is evidence that MeCP2 is phosphorylated site-specifically following synaptic firing to alter DNA binding affinity [100], perhaps to transiently and locally relax chromatin structure to promote gene expression. It is not clear to what degree these non-mutually exclusive mechanisms function in neuronal maintenance, but the concepts might have more general applicability. The idea that the replacement of H1 with a molecule that retains the chromatin compaction properties of the linker histone but confers additional responsiveness to internal or external stimuli is especially attractive.

4.3. H1 is underrepresented at transcriptional start sites

Poly(ADP-ribose) polymerase-1 (PARP-1) is a member of a large family of nuclear proteins that utilize NAD^+ as a substrate to catalyze the covalent attachment of negatively charged ADP-ribose units onto a variety of nuclear proteins, a process termed PARylation [101,102]. PARP-1 has been implicated in a wide variety of chromatin-based processes including DNA repair, stress management and transcription [103]. PARP-1 may also function as a chromatin architectural protein. Early *in vitro* studies showed that PARP-1 binds to nucleosomes in a position and manner similar to that of H1 to promote chromatin compaction [104,105]. This binding requires the DBD domain and the catalytic domain of PARP-1 (Fig. 2), although PARP-1 catalytic activity is not required. While H1 and PARP-1 were both shown to compete for binding to nucleosomes *in vitro*, the consequences *in vivo* appear to be quite different. A genome-wide analysis of occupancy revealed a reciprocal pattern of binding at actively transcribed promoters, in that H1 is relatively depleted and PARP-1 is relatively enriched [106]. Further studies showed that shRNA-mediated reduction of PARP-1 levels lead to reduced expression from selected genes concomitant with increased occupancy of H1 at or near the promoter of these genes. Additional observations lead to the view that PARP-1 might function to exclude H1 from the promoter region of active genes to promote an open chromatin conformation conducive to transcription [107]. Part of this process was attributed to PARP-1 PARylating and inhibiting the activity of the histone demethylase KDM5B, resulting in the retention of the activating epigenetic mark H3K4me3 at active promoters. However, treatment of cells with compounds that inhibit PARP-1 catalytic activity did not affect the exclusion of H1 from promoters, implying that this occurs upstream. Although most of the assays employed ChIP protocols, which do not directly provide information on molecular dynamics of binding, it is likely that relative concentrations and affinities of these two proteins for nucleosomal substrates are important contributing factors to the functional outcomes. Interestingly, treatment with the phorbol ester TPA, an activator of the protein kinase A signaling pathway, resulted in the repression of target genes that were also repressed by PARP-1 knockdown. TPA treatment was shown to promote PARP-1 release, resulting in increased H1 binding, decreased PolII binding, and reduced levels of H3K4me3 at these promoters. Thus, a possible

mechanism of the signaling pathway may be to alter a dynamic competition between H1 and PARP-1.

5. The case for H1 chaperones

Histone chaperones are a class of histone-binding proteins that are involved in a number of functions, which include histone transport and the promotion of efficient assembly and disassembly of histones onto nucleosomes [108]. Some chaperones are also involved in the regulated site-specific exchange of specific histone variants to mediate processes such as DNA transcription and repair. The substrates of most of the well characterized chaperones are core histones and the evidence for linker histone chaperones is relatively scant. This may be simply because the linker histones are inherently more mobile than core histones and do not require accessory proteins for the exchange process. However, the presence of proteins that might bind H1 variants in the off chromatin state, especially if this process could be regulated, would constitute a useful means to regulate chromatin conformation either locally or globally. Here we will review the data for several putative linker histone chaperones and their potential physiological functions. One of the most heavily studied linker histone chaperones is the nuclear autoantigenic sperm protein, or NASP [109]. There are two forms of NASP, both of which have been found to associate with H1 [110]. In somatic cells sNASP is critical for cell cycle progression, with alterations in NASP expression compromising cell growth and development, suggesting that sNASP functions primarily in the deposition of H1 onto DNA following replication [111,112] Another putative linker histone chaperone that has been the target of multiple studies is the nucleosome assembly protein 1 (Nap1) [113,114]. Nap1 has the ability to open the chromatin fiber *via* the reversible removal of the linker histone [115]. Interestingly, glutamylation of Nap1 is necessary for both proper linker histone dynamics and deposition of the linker histone onto chromatin [116]. This marks one of three studies which have attempted to decipher the importance of linker histone chaperones as they pertain to H1 mobility kinetics. The second involved the abundant mammalian acidic nuclear protein prothymosin α, one of the best known linker histone chaperone candidates [117,118]. Using permeabilized cells it was recently shown that prothymosin α can facilitate displacement and deposition of H1 onto native chromatin templates [119]. Additionally, siRNA-mediated knockdown of prothymosin α greatly slows linker histone kinetics. The third study attempting to correlate H1 mobility with chaperone activity involved Template Activating Factor-1 (TAF-1) [120]. In those experiments it was discovered that overexpressing TAF-1 enhanced linker histone mobility, facilitating H1.1 release from chromatin. It was also suggested that because TAF-1 preferentially binds to the C-terminal domain of H1 and prothymosin α binds to the globular domain [117] a complex between TAF1 and prothymosin α could exist. Further studies will be needed to clarify the role of histone chaperones in facilitating H1 exchange.

6. Concluding remarks

The primary function of the histones is to condense and package DNA to fit into the nucleus while maintaining a level of accessibility necessary for DNA-mediated processes such as transcription, replication, and repair. The core histones form an octamer, around which is wrapped 147 bp of DNA [3]. While the DNA within the nucleosome core exhibits dynamic properties [15], binding is significantly stable such that a given sequence will remain associated with a particular octamer for a significant duration, perhaps hours, days, or longer. The extended association of DNA with the octamer makes the core histones the ideal candidates for the epigenetic marks that constitute the histone code. In contrast, H1 exhibits a "dynamic" interaction with chromatin that is manifested as a behavior in which any single H1 molecule resides at a particular location for about a minute or so before dissociating and rebinding to a new location [1,2]. We suggest that linker histones have evolved to interact with the DNA between nucleosome cores to contribute to the establishment of an equilibrium that balances the need for accessibility with the need for condensation.

Local modifications of linker histones to alter their binding properties is a feasible mechanism to regulate gene expression in some instances [121]. However, we propose that the network of interacting architectural proteins plays a more passive role, essentially establishing a chromatin landscape based on the balance between the opposing needs of compaction and accessibility. Anthropomorphically speaking, the onus would lie more on trans-acting factors to interact within this landscape to direct functional outcomes, and we describe three different ways this might be accomplished.

While we have ascribed a somewhat passive role to the linker histones and the proposed dynamic chromatin network, this does not imply that it cannot be regulated. Global modulations of chromatin structure involving H1 dynamics are clearly involved in processes such as development, differentiation and the maintenance of plasticity in pluripotent cells [43,45,122]. Epigenetic marks on core histones have been said to confer "memory" to chromatin. In contrast, linker histone dynamics have been linked to re-programming of somatic cells to pluripotency [123] essentially allowing chromatin to "forget". Thus it is somewhat surprising in that H1 dynamics are also implicated in learning consolidation in mice [124].

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