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# Histone chaperones in nucleosome eviction and histone exchange

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## Summary

The recent two years have led to the realization that histone chaperones contribute to the delicate balance between nucleosome assembly and re-assembly during transcription, and may in fact be involved as much in histone eviction as they are in chromatin assembly. Recent structural studies (in particular, the structure of an Asf1 – H3/H4 complex) have suggested mechanisms by which this may be accomplished. The incorporation of various histone variants into nucleosomes has diverse effects on nucleosome structure, stability, and the ability of nucleosomal arrays to condense into chromatin higher order structures. It is likely that these seemingly independent ways to modify chromatin structure are interdependent.

## Introduction

The past two years have brought advances in our understanding of the roles of two rather different modulators of chromatin structure: histone variants and histone chaperones. Chromatin is composed of nucleosomes, which consist of an octamer of histones around which 147 base pairs of DNA are wrapped [1]. Histone variants are non-allelic isoforms of major-type histones; they affect nucleosome and chromatin structure and stability, thereby regulating access to DNA. Members of the diverse class of histone chaperones have long been dismissed as chaperones in the true sense of the word: proteins with ‘the specific task of preventing improper interactions between inappropriate partners’. Recently, histone chaperones have been implicated in histone removal and exchange in addition to their traditional role of aiding nucleosome assembly, and their role in maintaining chromatin structure and dynamics in non-replicating cells is increasingly recognized. Histone chaperones and histone variants are tightly interconnected at the functional level, in that histone variants may either favor or disfavor the action of histone chaperones through modulating nucleosome stability or higher order structures. Various histone chaperones will have different relative affinities for different histone variants, and this will affect the efficiency by which histone variants are incorporated into or removed from chromatin at any given location by any given factor. The molecular mechanisms for histone chaperone – mediated nucleosome disassembly and histone exchange are only now being elucidated. Much progress has been made regarding the biological function of histone chaperones and histone variants, and this has been covered in several recent reviews [2] [3] [4]. Here we focus on new developments from the past two years that merge our increased understanding of the effect of histone variants on chromatin structure and stability with structural and functional studies of various histone chaperones.

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## Histone variants modulate the stability of nucleosomes and nucleosomal arrays

### Nucleosome and chromatin structure

Numerous reviews have been written about the structure of the nucleosome (e.g. [5]). Of importance to the disassembly and reassembly of nucleosomes are the following properties. First, the $(H3\text{-}H4)_2$ tetramer organizes the central ~ 80 base pairs of DNA, whereas the peripheral ~ 40 base pairs of DNA are bound more loosely by the H2A-H2B dimers, with only a little contribution from the αN helix of H3 to organize the penultimate 10 base pairs of DNA at the entry and exit point. Second, the $(H3\text{-}H4)_2$ tetramer itself is held together via a tight network of hydrogen bonds and salt bridges that are confined to a small region defined by a four-helix bundle structure. Third, the H2A-H2B dimer makes intimate contacts with both arms of the W-shaped $(H3\text{-}H4)_2$ tetramer. These contacts involve the docking domain of H2A which interacts with the C-terminal β-strand in H4 and parts of H3 on one side, and a four-helix bundle between the histone fold regions of H4 and H2B (Figure 1). A very minor contact made by the H2A L1 loops exists between the two H2A-H2B dimers [6]. Even in sum, the interactions linking H2A-H2B dimers to the $(H3\text{-}H4)_2$ tetramer cannot persist in the absence of DNA, and thus the histone octamer is not stable under physiological conditions.

Given the tight interaction between H2A and H2B and between H3 and H4 to form the respective histone fold dimers, it is likely that these are the minimal units of nucleosome assembly and disassembly. Nucleosome assembly is a stepwise process in which H3/H4 is deposited onto the DNA, followed by deposition of H2A/H2B. There are two fundamental ways by which the stability of the nucleosome may be altered: by altering the interface between the H2A-H2B dimers and $(H3\text{-}H4)_2$ tetramer, and / or by altering the histone / DNA interface. Chromatin higher order structure is affected by amino acid variations on the histone octamer surface and in the histone tails that mediates nucleosome – nucleosome interactions to form secondary and tertiary chromatin assemblies [7] [8] [9]. Thus, modulation of nucleosome and chromatin structure may be achieved through the incorporation of histone variants (reviewed in [4]).

### H2A variants

H2A variants are particularly divergent in regions that are involved in histone – histone contacts or in surface residues [10]. Consequently, different effects on nucleosome and chromatin structure have been observed depending on the histone variant. For example, H2A.Z stabilizes the nucleosome [11] [12] and higher order structures [13] *in vitro*. Histone octamers reconstituted with macroH2A (a histone variant associated with repressive chromatin) appear to be stabilized [14]; and chaperone-mediated removal of macro-H2A-H2B dimers as well as remodeling of nucleosomes is disfavored [15]. H2A.Bbd (a H2A variant generally associated with transcriptionally active chromatin) lacks the carboxy-terminal tail as well as a signature acidic surface. H2A.Bbd incorporation results in a less compact nucleosome in which DNA may be more amenable to transcription factor binding [16,17]. These nucleosomal arrays have a more extended conformation and H2A.Bbd inhibits the folding of 30-nm fiber like structures [9].

### H3 variants

Histone H3.3 exhibits a distinct expression and localization pattern despite the fact that only four amino acids differ from major-type H3.1. Despite these minor differences, the stability of nucleosomes containing H3.3 is reduced [18]. CenH3, the centromeric version of H3, is perhaps the most enigmatic histone variant in that it may form non-canonical nucleosomes. Three independent studies (mostly based on *in vivo* experiments) have shown that yeast CenH3 (Cse4)

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forms a centromere-specific nucleosome together with H4 and the non-histone protein Scm3 [19] [20] [21]. In fact, it has been suggested that Scm3 may be a Cse4-specific histone chaperone [21]. There is also evidence (mainly from *in vivo* protein – protein crosslinking) that cenH3 containing nucleosomes in *Drosophila* cell lines can exist as 'hemisomes', that is, that they consist of only one copy each of cenH3, H4, H2A, and H2B [22]. Clearly, a biophysical analysis of these intriguing variant nucleosomes and their arrays will be of high interest. Already looking beyond a basic characterization of CenH3-containing chromatin, a landmark study from the Cleveland lab describes an entire complex (containing several newly described proteins) that is associated with human CenH3. Intriguingly, the histone chaperones FACT and nucleophosmin-1 are also stably recruited to CenH3 nucleosomes [23].

### 'Mixing and matching' of histone variants

There are two copies of each histone in a nucleosome (Figure 2), and many different histone variants and replacement histones exist in the cell. *A priori*, there is no reason to assume that variants of H2A and H3 cannot co-exist in a single nucleosome, and likewise, there is no good reason to assume that both H2A in one nucleosome are variants. For example, histone H3.3 was found to co-localize with H2A.Z *in vivo* [18]. These nucleosomes appear to be destabilized to the extent that they could not be isolated from native chromatin without crosslinking [18]. Thus, the presence of H2A.Z may potentiate the destabilizing effects of H3.3. Preferential removal of H2A.Bbd-H2B dimers from nucleosomes also containing H3.3 has been demonstrated *in vitro* [24]. Human CenH3 nucleosomes are more likely than their major-type H3 counterparts to contain macroH2A and H2A.Z [23]. Lastly, while *in vitro* data demonstrating that macroH2A preferentially teams up with a major-type H2A within a single nucleosome remain to be confirmed *in vivo*, these data suggest an additional degree of freedom for altering the chemical composition and thus stability of nucleosomes [14].

## Histone chaperones in nucleosome assembly and disassembly

The absolute stability of the nucleosome under any defined condition *in vitro* is unknown. *In vivo*, histones are turned over *in vivo* in the absence of replication, and many histone variants are introduced into chromatin in a replication-independent manner. Consistent with its more peripheral location on the nucleosome, H2A-H2B dimer exchange is more rapid than that of H3-H4 [25] [26]. The complete eviction of nucleosomes at promoter regions appears to be a widely occurring phenomenon in yeast, and perhaps also in metazoans [27] [28]. Histone chaperones have recently attracted much attention because of their involvement in these processes (reviewed in [29]). Previously perceived as simple histone binding proteins that are in some peripheral way involved in histone transport into the nucleus and in nucleosome assembly, histone chaperones are now emerging as proteins that help maintain a delicate balance between nucleosome assembly and partial or complete disassembly (Figure 2). It is not known whether the removal of histones from DNA occurs by the same mechanism with which they are deposited. However, it appears likely that many histone deposition factors will promote to some extent the reverse reaction of partial or complete nucleosome disassembly. This has been directly shown for NAP1, both *in vitro* and *in vivo* [30] [31] [32] [33], FACT (*in vitro* and *in vivo*; reviewed in [34]); Asf1 (*in vivo*; [35]), and nucleolin (*in vitro*; [36]).

### Chaperone – histone interactions and implications for nucleosome dissociation

A mechanistic understanding of nucleosome disassembly requires a detailed knowledge of nucleosome thermodynamics and chaperone – histone interactions. Progress is being made on both fronts. The analysis of nucleosome thermodynamics has been daunting, due to the complexity and high stability of the system under investigation. Developments in single molecule approaches appear the most promising [37] [38] [39].

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Histone chaperones have traditionally been grouped into chaperones that are 'specific' for H2A/H2B (e.g. NAP1, FACT, Chz1, nucleophosmin, nucleoplasmin, nucleolin), or H3/H4 (e.g. Asf1, Spt6, HIRA, or CAF1); (recently summarized in [2] [3]). In some instances chaperones are indeed highly specific. Chz1, for example, appears to preferentially bind H2A.Z/H2B over H2A/H2B [40]. On the other side of the spectrum is NAP1, which binds all histones and even H1 rather non-discriminately, yet with high affinity [41]. To our knowledge no definitive measurements exist on the affinities or association/dissociation rate constants for any chaperone binding to any histone; these are required as an important piece of the puzzle to establish the mechanism for chaperone – mediated nucleosome dissociation and assembly.

Much progress has been made in the structure determination of a variety of histone chaperones. The structures of several variants of nucleoplasmin ([42] and references therein), NAP1 and the related SET/TAF-1 [43] [44] (all putative H2A/H2B chaperones) are now known, as is the structure of the H3-H4 chaperone Asf1 [45]. The structures of HIRA and the histone binding subunit of CAF-1 have been predicted through homology modeling [46,47]. These studies have revealed no similarities in the overall fold or oligomerization state of histone chaperones. However, common to the four types of structures is a four-stranded β-structure (Figure 3). For Asf1, this region has been shown to directly interact with histones ([48], see below). Mutational studies have implicated the β-domain of SET/TAF-1 in histone binding [44], suggesting the possibility that this conserved β sheet may be the primary histone recognition motif for the NAP1 family also.

At this time, the only detailed structural information for how a histone chaperone interacts with histones is available for the Asf1 - H3/H4 or H3 complex [48–50]. These structures have been very rewarding in that they provide intriguing insights into the mechanism of nucleosome disassembly. *In vitro* and *in vivo* evidence that Asf1 binds a heterodimer of H3/H4 (and not, as previously assumed, a $(H3\text{-}H4)_2$ tetramer) [51] [52] [53] were confirmed by x-ray crystallography, and the structural basis for these observations became immediately obvious. Asf1 binds the C-terminal α3-helix of H3, preventing the formation of the $(H3\text{-}H4)_2$ through the four-helix bundle (Figure 4). *In vitro* and *in vivo* mutagenesis studies have shown the relevance of these binding regions for Asf1 chaperone activity [48,49]. The C-terminal β strand of H4, which in the nucleosome forms a two-stranded parallel β sheet with the docking domain of histone H2A [1] forms an antiparallel β sheet with the last β strand of Asf1. Based on these observations, an intriguing "strand-capture" model for the disassembly of histone H3-H4 tetramer from the nucleosome was proposed [48]. Asf1 binding to the C terminus of H4 may aid the release of the H2A-H2B dimer during the initial phase of chromatin disassembly, and additional interactions between Asf1 and H3 may cause the splitting of the H3-H4 tetramer into Asf1 bound H3-H4 dimers. This intriguing mechanism needs to be tested *in vitro*. Despite the well-documented role of Asf1 in nucleosome eviction *in vivo* [35]; [54] and references therein), there is no *in vitro* evidence that Asf1 can directly mediate nucleosome disassembly, either alone or together with ATP-dependent remodelers or H2A/H2B chaperones. It should be pointed out that the concept of the H3/H4 dimer as the intermediate for nucleosome assembly has been proposed previously [55].

At the present time, no structures are as yet available for any chaperone - H2A/H2B complex, and it is unknown how much of the structure of the H2A-H2B dimer as it exists in the nucleosome is retained when forming a complex with a chaperone. While it is possible that H2A/H2B chaperones promote removal of the H2A-H2B dimer from the nucleosome by simply stabilizing its 'off-state' (which is perhaps promoted by the transient unraveling of the DNA [38]), this is likely not the case at least for yeast NAP1 [30]. The C-terminal acidic domain of yNAP1 (which is completely disordered in the crystal structure) is essential for the ability of yNAP1 to disassemble nucleosomes, even though the truncated version binds histones with the same affinity as the full length protein and is sufficient for assembly [30]; G. Downing, T.

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Sakurai, and A.J. Andrews, unpublished results). Interestingly, acidic stretches of varying lengths are found in numerous histone chaperones, and many (including metazoan NAP1) are post-translationally modified by the addition of 8–10 polyglutamate residues [56].This suggests the exciting possibility that the diverse chaperone activities (Figure 2) could be regulated by posttranslational modifications.

### Histone variant specific eviction and assembly

To date, there is not much quantitative or structural data to support the notion that histone chaperones *per se* preferentially facilitate assembly or disassembly of certain variant-containing nucleosomes. Each histone chaperone and variant nucleosome must be evaluated *in vitro* for the affinity of chaperone-histone complexes, for the stability of the nucleosome, and for the mechanism by which each chaperone promotes histone assembly / disassembly. *In vivo*, the relative amounts of variant to major-type histone, and the relative amount of individual chaperones at any given time must also be investigated, as the concentration of free and histone – bound chaperone also will affect exchange rates. It is already evident that generalizations will not be possible. For example, members of the NAP1 family of chaperones do not even appear to distinguish between H3/4 and H2A/H2B complexes, whereas the structure of the Asf1-H3/H4 complex suggests that Asf1 cannot bind H2A/H2B through similar interactions. Human ASF1 co-purifies with both H3.1 and H3.3. HIRA and CAF1 are specifically required to mediate H3 replacement with H3.3 [55]. Human RbAp46/48 assemble CenH3 into chromatin *in vitro* [57], however, Scm3 may also be involved [21]. Finally, Chz1 shows a preference for H2AZ-H2B over H2A-H2B and cooperates with the SWR1 complex in the exchange of H2A for H2A.Z [40] [58]. How the subtle sequence differences between histone variants and their major-type counterparts are recognized by the various histone chaperones remains to be determined through structural studies and through rigorous quantitative analysis of the individual interactions.

## The promotion of the histone chaperone

It appears that the role of the histone chaperone has been upgraded from that of a passive bystander to that of an active player in the maintenance of chromatin dynamics. Many histone chaperones at least on occasion team up with with ATP-dependent chromatin remodeling factors. For example, RSC disassembles a nucleosome in the presence of the histone chaperone NAP1, but not ASF1, suggesting the functional specificity of the individual histone chaperones [59]. SWI/SNF-dependent nucleosome remodeling is enhanced by nucleolin [36], and CHD1 cooperates with NAP1 in nucleosome disassembly at promoters and in coding regions [32]. SWR1, a complex that exchanges histone H2A.Z into assembled chromatin co-opts either NAP1 or Chz1 to either facilitate H2A-H2B dimer removal or deliver the variant histone complex [58]. Physical and functional links between chaperones and HATs have also been described. There is evidence that NAP proteins are functional components of the p300 co-activator complex [60] and augment p300 enzymatic activity. The NAP1 homologue TAF1/SET is a subunit of the INHAT complex, a multiprotein complex that inhibits the HAT activity of p300 and PCAF [61]. Another recently discovered NAP1 family member, Vps75, as well as Asf1, interact with the novel fungal histone acetyl transferase Rtt109 and appear to be required for HAT activity [62] [63].

This brings up intriguing questions: Exactly what is the role of chaperones in ATP-dependent remodeling? Do they temporarily safe-keep histones, or do they facilitate remodeling itself? Do they alter the outcome of remodeling? Do histone chaperones participate in substrate presentation to the HAT, or do they have novel functions as regulators of activity? Do they team up with other chromatin modifiers? How promiscuous are these ‘chaperones’ really? All of these questions need to be addressed at the mechanistic level, and it will be much fun to sort all of this out. Who would have thought that a chaperone could be the life of the party?

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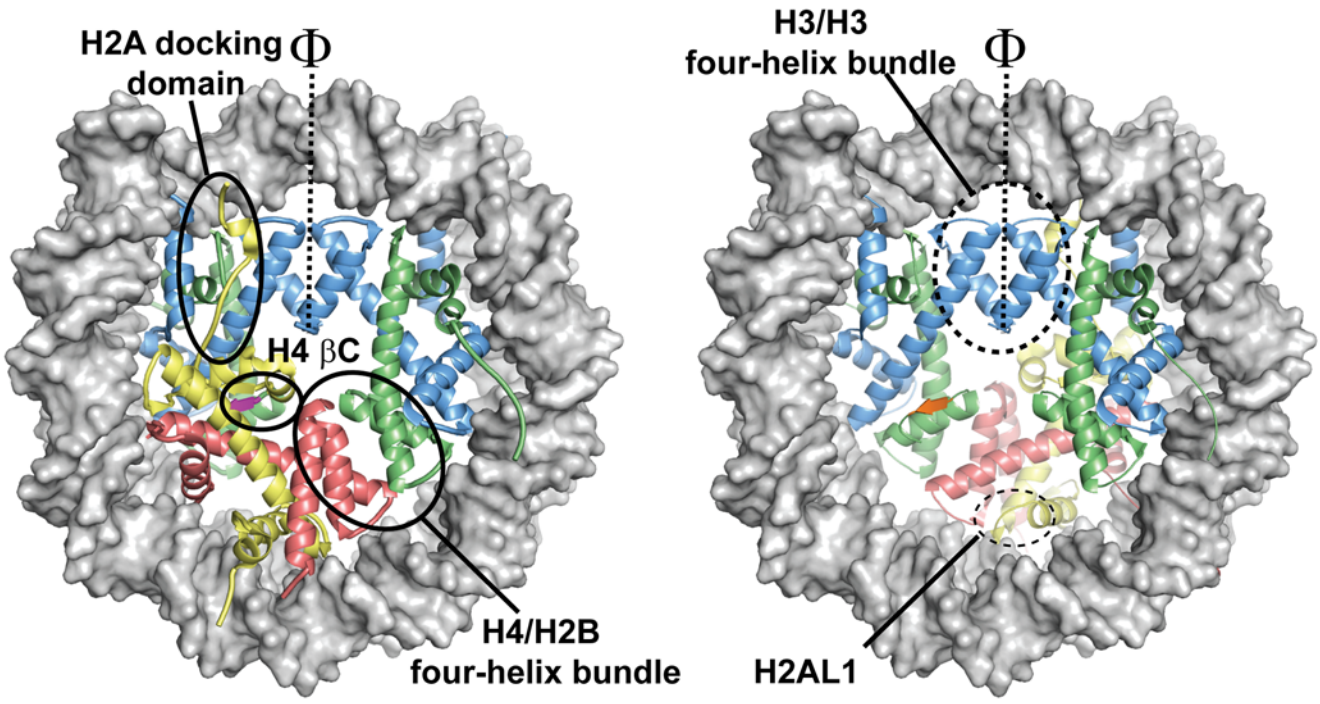


**Figure 1. Structure of the nucleosome (pdb code 1aoi)**

DNA is shown in grey, H2A in yellow, H2B in red, H3 in blue, and H4 in green, respectively. Only one of the two H2A-H2B dimers is shown in each panel for clarity. The molecular two-fold axis dissecting the H3 four-helix bundle is indicated by a dashed line. Regions of interaction between the H2A-H2B dimer and the $(H3\text{-}H4)_2$ tetramer are indicated by solid ovals (left panel), whereas homotypic interactions between H3 or H2A are indicated by dashed ovals (right panel).

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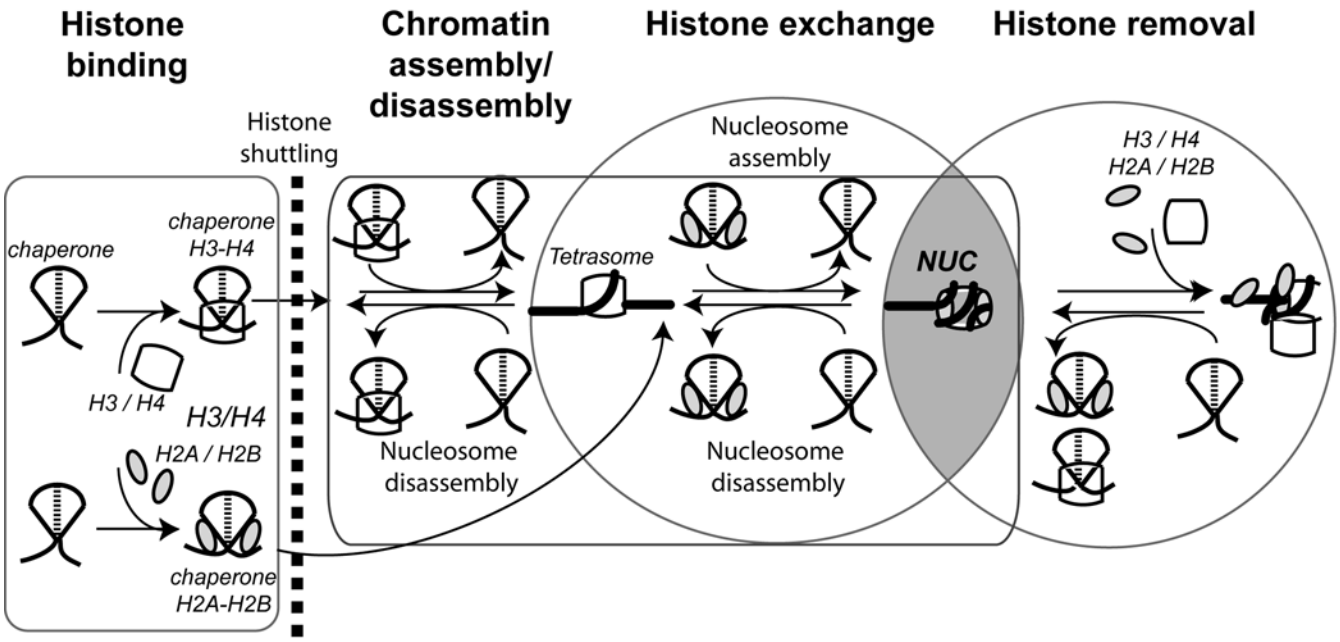


**Figure 2. A summary of potential histone chaperone functions**

The various functions of a chaperone are determined by the relative affinity of a chaperone for histones compared to the relative affinity of a histone bound to DNA. Both can be varied through posttranslational modifications of histones and / or chaperones, through sequence variations found in histone variants, and through the presence of other factors.

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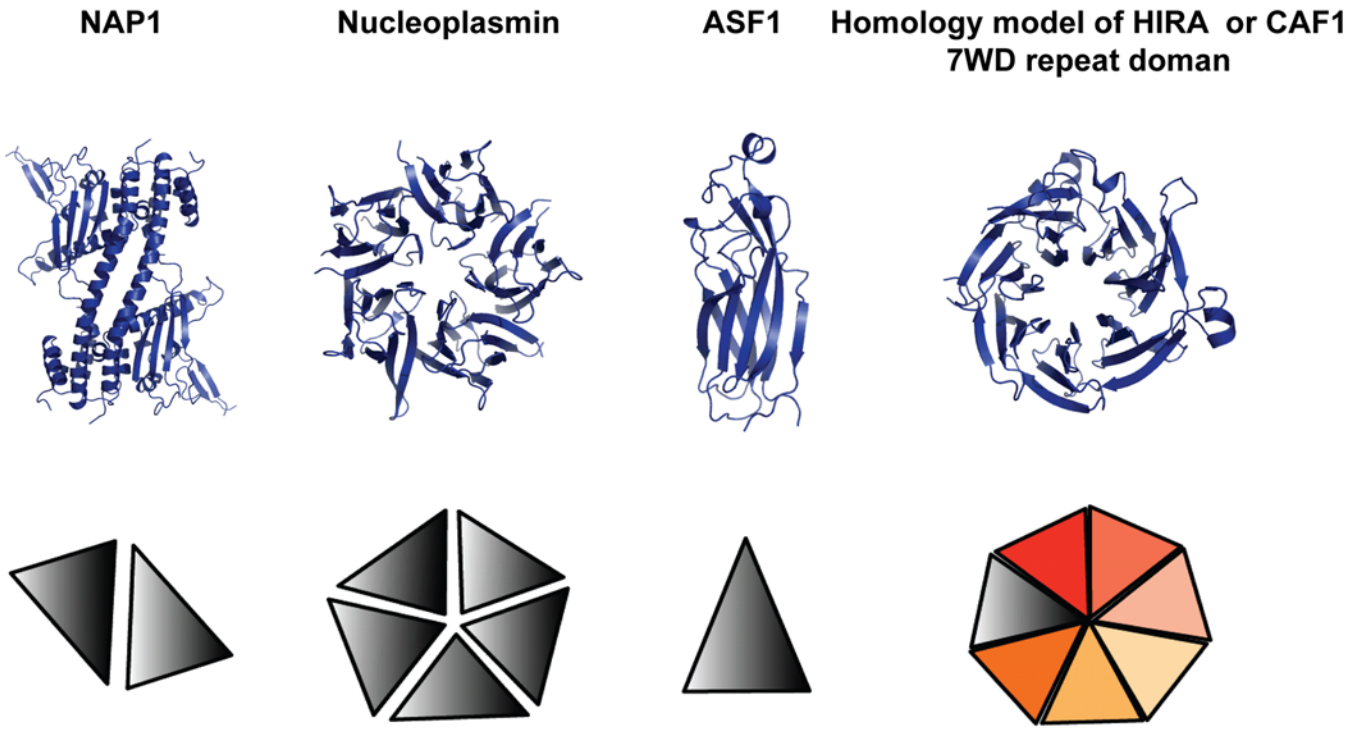


**Figure 3. Histone chaperones share a β sheet motif, but otherwise exhibit no similarities in tertiary or quarternary structures**

NAP1 (2ayu), Nucleoplasmin (1k5j)), Asf1 (1roc) and a homology model (1sq9) of HirA or CAF1 are shown as a ribbon diagram. Each monomer containing a four-stranded β sheet is represented as a gray triangles (bottom panel). In the homology model, the predicted 7 repeat domains within single peptideare shown with different colors and the best-matched 4 beta-sheet motif is in gray.

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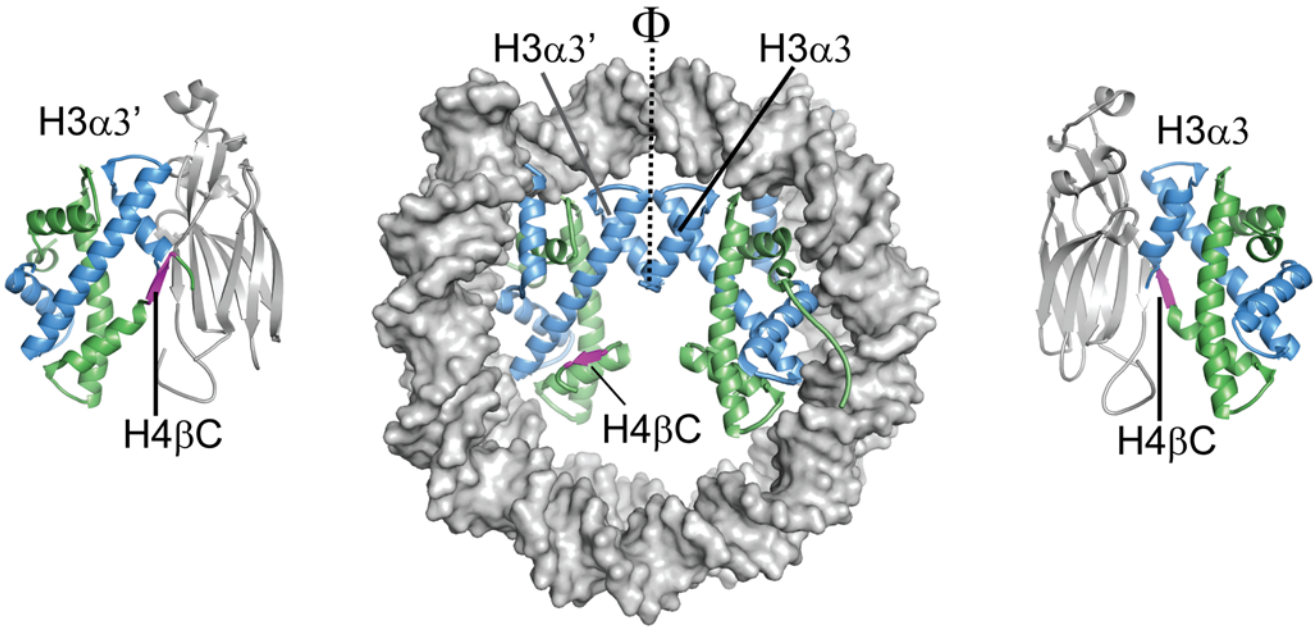


**Figure 4. The disassembly of the DNA-bound $(H3\text{-}H4)_2$ tetramer by Asf1**

The C-terminal β strand of H4 (magenta) undergoes a rotation to joins a β-sheet in Asf1. Tight interactions between the H3 α3 and α2 helices are incompatible with the four-helix bundle structure. The H2A-H2B dimers are omitted for clarity, and indeed may have to be removed prior to Asf1-mediated $(H3\text{-}H4)_2$ tetramer disassembly. Note that none of the regions of H3/H4 that interact with the DNA are occluded by Asf1.