

Interactions of Histones with DNA: Nucleosome Assembly, Stability, Dynamics and Higher Order Structure

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1. Introduction

The genome of a human cell comprises 6×10^9 DNA base pairs corresponding to a DNA chain with a total contour length of about two meter. Free in solution a DNA duplex of this length would occupy the volume of a sphere with a diameter of approximately 400 μm as estimated from its calculated radius of gyration. This amount of DNA is packaged into a nucleus with a typical diameter of 10-20 μm . The required compaction of the genome is obtained via complexation of the DNA with small strongly positively charged proteins, the histones, into a large nucleoprotein complex that is referred to as chromatin [1]. At the same time the genetic information has to remain accessible for DNA binding factors involved in processes like replication, transcription, repair and recombination. Thus, the interaction of histones and DNA has to mediate between these two apparently contradicting functions in a dynamic manner. It represents an important regulatory factor for all processes that require direct access to the DNA. Histones are among the evolutionary most conserved proteins [2]. They can be classified into five groups, namely the four core histones H2A, H2B, H3 and H4 and the linker histone H1. In mammals, each of these classes except H4 is subdivided into several subtypes as well as the so called histone variants or substitution histones [1,3]. Two copies of H2A, H2B, H3 and H4 constitute a histone octamer complex that is the protein core around which 146 or 147 base pairs of DNA are wrapped in 1.67 turns of a left-handed superhelix. This nucleoprotein complex is termed the nucleosome and is the main building block of eukaryotic chromatin. The structure of the free histone octamer and that of the nucleosome complex have been determined at atomic resolution by x-ray diffraction [4-8]. Each nucleosome is separated by 10-60 bp of "linker" DNA, and the resulting nucleosomal chain of ~ 10 nm in diameter is folded into more condensed fiber of ~ 30 nm diameter, which itself forms higher order structures. The linker histone H1 or its subtype H5 in avians, organizes the internucleosomal linker DNA [9-12]. DNase I cleavage assays showed that binding of linker histones to a nucleosome protects an additional 20 base pairs of DNA flanking the nucleosome structure [13-15]. The complex of nucleosomes, linker histone and the interacting ~ 169 bp of DNA is referred to as the chromatosome.

2. Histones

2.1 Core histones

The octameric complex of the histone H2A, H2B, H3 and H4 represents the standard protein core of the nucleosome. Several subtypes of the canonical core histones H2A, H2B, and H3 have been described with differences in 2 or 3 amino acids. These are referred to as the H2A subtypes H2A.1 and H2A.2, the H2B subtypes H2B.1, H2B.2, and H2B.3, and the H3 subtypes H3.1 and H3.2 [16]. It is noted that the distinction between histone subtypes and histone variants/replacement histones (see below) is somewhat arbitrary. It is not clear whether the above subtypes are truly equivalent or exhibit distinct activities in the cell, and for H3.1 and H3.2 differences in their expression and posttranslational modification pattern have been reported that might be relevant establishing functionally different chromatin states [17]. For H4 only a single 102-amino acid sequence has been found in mammals but multiple gene copies exist for all the canonical core histones in the human genome [3,18]. Most of these are clustered at chromosome 6p21 and 6p22 (NIH histone sequence database, <http://research.nhgri.nih.gov/histones/web/chrmap.shtml>). Their expression is strictly regulated and restricted to the S-phase of the cell cycle.

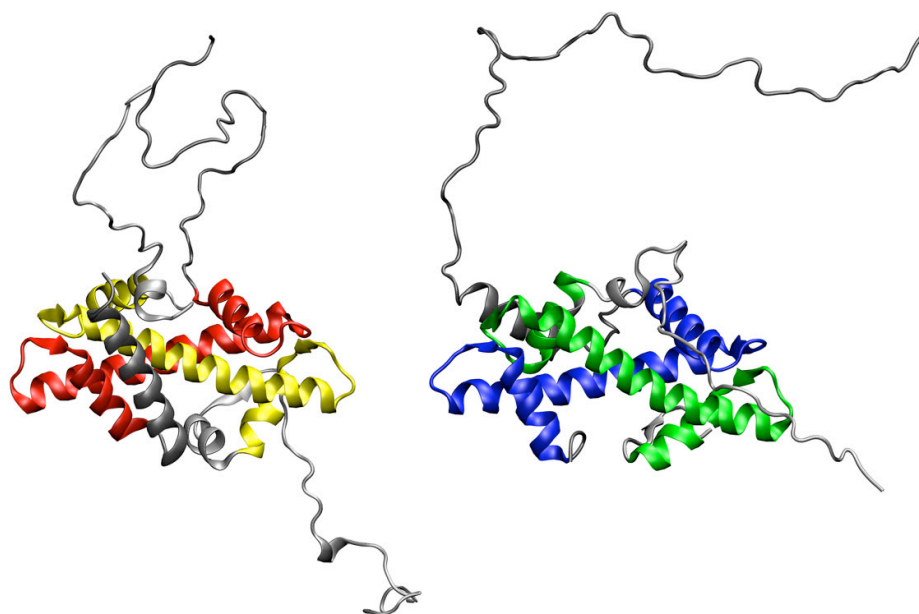


Figure 1. Histone fold and histone-histone interactions. The (H2A·H2B) dimer (left panel) and a (H3·H4) dimer (right panel) are shown. Histone proteins are colored yellow for H2A, red for H2B, blue for H3, green for H4.

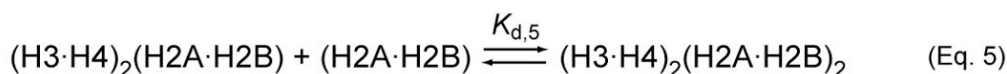
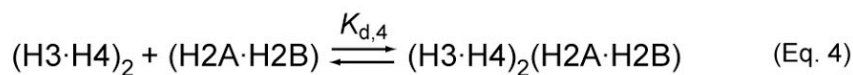
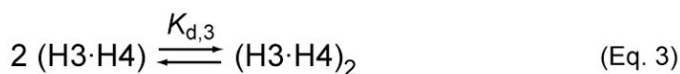
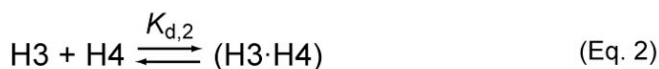
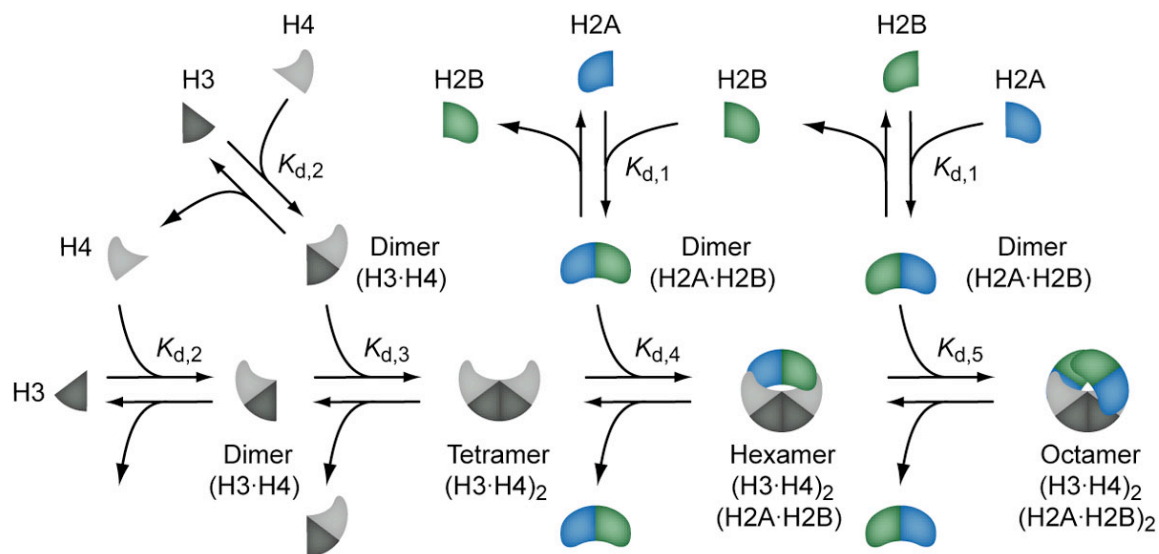


Figure 2 Reaction scheme for assembly of the histones octamer. The equilibrium for each step is described by the dissociation constant K_d . The designation (H2A·H2B) represent the heterodimer between HA and H2B, (H3·H4) the heterodimer between H3 and H4, (H3·H4)₂ is the tetramer, (H3·H4)₂(H2A·H2B) the hexamer and (H3·H4)₂(H2A·H2B)₂ is the octamer complex.

Individual core histones tend to form large and unspecific aggregates at physiological ionic strength. At very low ionic strength they are soluble in limited amounts and adopt a mostly unfolded random coil conformation [1]. In contrast, in the histone octamer each of the core histones adopts a similar secondary structure in its globular domain, the “histone fold” that mediates histone-histone interactions. The histone fold is a three-helix motif which heterodimerizes by forming a handshake like structure between H3 and H4 as well as between H2A and H2B [19] (Fig. 1).

Table 1. Thermodynamic parameters for histone-histone complexes

Reaction	ΔG (kcal mol ⁻¹)	K_d	$\Delta\Delta G/\Delta I$ (kcal mol ⁻¹ M ⁻¹)	Ref.
H2A + H2B \rightleftharpoons (H2A·H2B)	-9.6 ± 1.4 (0.1 M salt)	9·10 ⁻⁸ M	-8.0	Eq. 1 ^a
H3 + H4 \rightleftharpoons (H3·H4)	-11.6 ± 1.2 (0.1 M salt)	3·10 ⁻⁹ M	-11.7	Eq. 2 ^b
2 (H3·H4) \rightleftharpoons (H3·H4) ₂	-6.9 ± 0.3 (0.1 M salt)	9·10 ⁻⁶ M	-2.0	Eq. 3 ^c
2 H3 + 2 H4 \rightleftharpoons (H3·H4) ₂	-30.6 ± 1.9 (0.1 M salt)	5.2·10 ⁻²³ M ³	-25.4	Eq. 2+3 ^d
(H3·H4) ₂ + (H2A·H2B) \rightleftharpoons (H3·H4) ₂ (H2A·H2B)	-7.7 (2.0 M salt)	2·10 ⁻⁶ M	≈1.5	Eq. 4 ^e
(H3·H4) ₂ (H2A·H2B)+(H2A·H2B) \rightleftharpoons (H3·H4) ₂ (H2A·H2B) ₂	-7.8 (2.0 M salt)	2·10 ⁻⁶ M	≈1.5	Eq. 5 ^e

Free energy values are given for the formation of histone complexes by the reactions depicted in Fig. 2. For the formation of the (H2A·H2B) and the (H3·H4) dimer data are included that are derived from measurements of the free energy for unfolding due to heating or the addition of denaturant. Thus, it is implicitly assumed that the isolated histones are mostly in an unfolded state. While this has been shown to be the case for low ionic strength some additional folding of the individual histones is likely to be present at 0.1 M salt concentrations [1].

^a Previously reported values were extrapolated to 100 mM salt to obtain -9.0 kcal mol⁻¹ [20], -11.2 kcal mol⁻¹ [21] and -8.7 kcal mol⁻¹ [22]. For the salt dependence of ΔG in the range of 0.1 to 1 M, values of $\Delta\Delta G/\Delta I = -9.8$ kcal mol⁻¹ M⁻¹ [22] and $\Delta\Delta G/\Delta I = -6.2$ kcal mol⁻¹ M⁻¹ [21] were averaged.

^b The ΔG value of -8.8 kcal mol⁻¹ determined at pH 4.5 [23] was extrapolated to pH 7.5 by assuming the increase of stability by ~2.8 kcal mol⁻¹ upon raising the pH as observed for (H2A·H2B) [22]. The value of $\Delta\Delta G/\Delta I$ was derived from measurements between 0.01 to 0.12 M salt [23].

^c Average value from -7.2 kcal mol⁻¹ [24] and -6.7 kcal mol⁻¹ [25]. The salt dependence was calculated from measurements at 0.1, 0.5 and 1 M [25].

^d The free energy of -28.4 kcal mol⁻¹ determined in 16 mM phosphate buffer [20] was extrapolated to be -30.6 kcal mol⁻¹ at 100 mM salt by using the salt dependence of $\Delta\Delta G/\Delta I = -25.4$ kcal mol⁻¹ M⁻¹ determined for the single reactions. The ΔG for the formation of the (H3·H4)₂ complex calculated as the sum of Eq. 2 and 3 is 2·(-11.6 kcal mol⁻¹) + (-6.9 kcal mol⁻¹) = -30.1 kcal mol⁻¹.

^e Values refer to macroscopic binding energies in the presence of 2 M NaCl [26,27]. As calculated previously the intrinsic/microscopic free energies for binding of the (H2A·H2B) dimer are -7.3 kcal mol⁻¹ for the hexamer and -8.2 kcal mol⁻¹ for the octamer indicating cooperativity of binding [1]. The salt dependence was estimated from the observed dissociation of 20 μM octamer upon lowering the salt concentration to 1.2, 0.8 and 0.6 M [28].

The core histones contain an unstructured segment of 20-35 residue at the amino-terminus that is rich in basic amino acids. Histone H2A is unique in having an additional ~37 amino acid carboxy-terminal domain that protrudes from the nucleosome. These histone 'tails' are targets of post-translational modifications and play an important role in modulating the interaction of histones with other chromosomal proteins and/or between nucleosomes [29]. In the absence of DNA the histone octamer complex is stabilized by high salt concentrations of ~2 M. Decreasing the salt concentrations to physiological levels (100 to 150 mM salt) or increasing the temperature promotes the dissociation into histone subparticles [26-28]. These include a (H2A·H2B) heterodimer, a (H3·H4) heterodimer and a (H3·H4)₂ heterotetramer. The reaction scheme for the (dis)assembly of the histone octamer is depicted in Fig. 2. The stability of these particles in terms of their free energy and their dissociation constant K_d can be estimated from previous work (Table 1). For the dependence of the free energy ΔG on ionic strength I an apparent linear relationship with the slope $\Delta\Delta G/\Delta I$ has been reported in previous studies at salt concentrations up to 1 M for the stability of the (H2A·H2B) dimer [21,22] and the histone (H3·H4) dimer [23]. Accordingly, a value of $\Delta\Delta G/\Delta I$ has been derived from the available data and represents the change of ΔG upon an increase of the salt concentration by 1 M.

2.2 Linker histones

Around 80% of the nucleosomes harbor a linker histone H1 or one of its variants, which sits near the entry/exit site of DNA in the nucleosome, organizing ~20 bp DNA flanking the nucleosomal core in a stem loop like manner [13,30,31]. The members of the H1 group of proteins show a rather conserved structure, consisting of a compact globular domain (GD) with a winged-helix fold [32,33] and a C-terminal domain (CD), which are surrounded by a short N-terminal and a longer C-terminal tail. It appears that only the globular domain of the linker histone is folded in the absence of DNA and only for this part of the protein a high resolution structure has been reported [32].

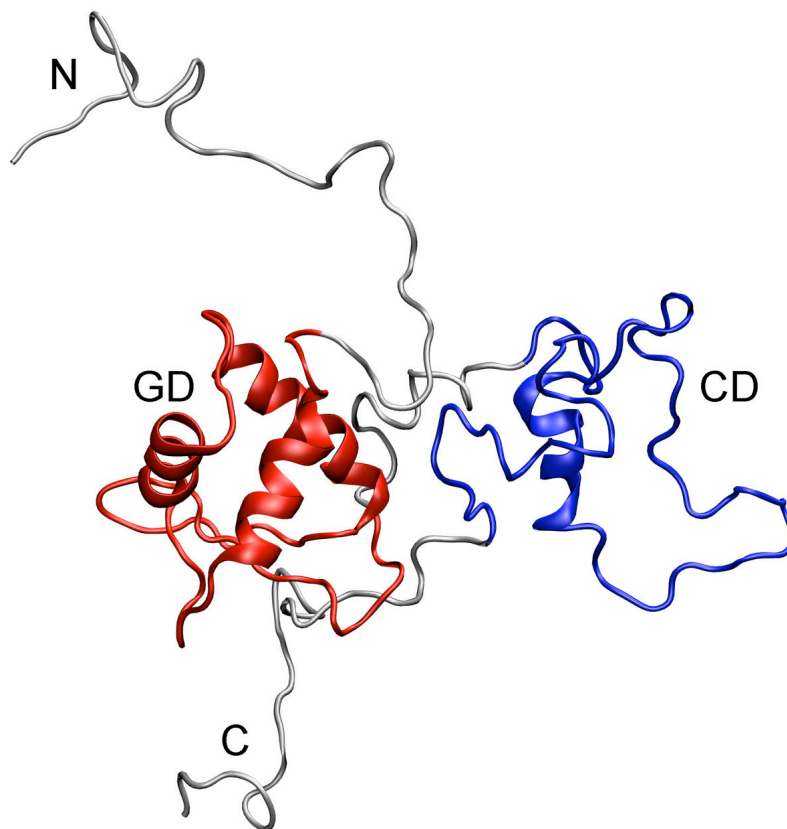


Figure 3. Energy minimized model structure of a H1 linker histone. The N-terminus, (N, residues 1 – 21), the globular domain (GD, residues 22 – 96), the C-terminal domain (CD, residues 110 -183) and the C terminus (C, residues 184 – 193) are indicated. The numbering refers to the sequence of H1.2 from rat with the CD structure derived from previous model building studies in its complex with DNA [34]. The globular domain corresponds to the protein fold determined in the crystal structure of the related linker histone H5 [32].

The linker histone H1 stabilizes the nucleosomal structure, reduces mobility of the nucleosome and guides higher-order chromatin folding [35-37]. Accordingly, the linker histone is found to accumulate in transcriptionally inactive regions [38], whereas transcriptionally active regions appear depleted of linker histone. Furthermore, the linker histone was shown to impede transcription *in vitro* [39]. Knock-out studies of H1 in mouse embryonic stem cells demonstrate large changes in chromatin structure with respect to global nucleosome spacing, local chromatin compaction, and decreased modification of certain core histone [40,41]. In these studies H1 affected transcription of only a small set of genes. These genes are also closely regulated by DNA methylation suggesting a connection between linker histone function and DNA modification

2.3 Histone Variants

Histone variants or substitution histones can replace the canonical core histones H2A, H2B, H3 and H4. They provide the possibility to generate a specialized chromatin environment for nuclear processes in certain regions of the genome [42-45]. Histone variants are summarized in Table 2 and include variants in H2A (H2A.Z, H2A.X, macroH2A, H2ABbd), H2B (H2BFWT, hTSH2B) and H3 (H3.3, CenH3). No histone variant for core histone H4 is known. A comprehensive review of histone variants is beyond the scope of the present report, and only those histone variants for H2A and H3 are described briefly in the following that are considered to be the most important. For histone H2A these are H2A.X, H2A.Z and macroH2A. The H2A.X histone is thought to play a role in DNA repair since it is phosphorylated at a characteristic C-Terminal SQ motif in response to the introduction of DNA double-strand breaks [46]. This modification appears to recruit a large set of additional factors that might mediate different DNA modifying activities [47]. H2A.Z moderately stabilizes the nucleosome [48,49] and is mostly associated with regions of transcriptionally active chromatin [50]. Its recruitment is conducted by the ATP-dependent chromatin remodeling complex SWR1 in yeast, which replaces H2A with the variant histones in nucleosomes *in vivo* and *in vitro* [51,52]. The overall structure of the H2A.Z nucleosome displays high similarity to the canonical nucleosome [53] with respect to the histone-fold domains and the DNA path on the nucleosome surface. The essential region for H2A.Z activity seems to lie in its acidic C-Terminal amino acid stretch [54], which might function as a protein binding site [55]. Finally, macroH2A has been related to repression of transcription by inhibiting transcription factor binding as well as remodeling of histones and their acetylation [56,57]. The variant is found to be enriched in the inactivated X chromosome [45,58,59]. It is characterized by a large C-terminal macro domain, that has ADP-ribose binding capacity [60]. The incorporation of this variant increases the stability of the nucleosome in agreement with its proposed function in transcriptional silencing [61].

Table 2. Histone variants

Canonical histone	Histone variant	Localization and function	Ref.
H2A	H2A.Z	mostly transcriptionally active chromatin; prevents spreading of heterochromatin into euchromatic regions	[50,62-64]
	H2A.X	involved in DNA repair via recruitment of double strand break repair complexes upon phosphorylation of H2A.X	[65-67]
	H2ABbd	associated with regions of H4 acetylation, excluded from inactive X-chromosome; reduces nucleosome stability	[68,69]
	macroH2A	accumulated at inactive X-chromosome; contains a large C-terminal macrodomain the could bind ADP-ribose	[58,60,70]
H2B	H2BFWT	located at telomeric sequences; inhibition of association with chromatin condensation factors	[71]
	hTSH2B	testis specific; possibly telomere-associated functions	[72,73]
H3	H3.3	transcriptionally active chromatin; derepression of genes	[74]
	CenH3	associated with centromeric DNA; essential for assembly and stability of kinetochores	[75-77]

The histone H3.3 variant is involved in gene regulation and marks active chromatin. It is very similar to the canonical H3 in the amino-acid sequence as only a few amino acids (4 in *Drosophila*) are changed [74]. However, both incorporation into chromatin and genome localization are very different from H3. Unlike its canonical counterpart, H3.3 is not deposited by the chaperone complex CAF1-ASF1 on the DNA but by the HIRA-ASF1 complex in a replication-independent manner [78]. The centromeric H3 variants (CenH3s) from different organisms, such as the mammalian CENP-A (centromere protein A) are constitutive components for centromeres [75,79]. CENP-A is required for recruitment and assembly of additional centromere specific factors such as Cen-C and guide proper centromere and kinetochore organization [80,81]. Furthermore, it has been described as an epigenetic mark of centromeric chromatin throughout DNA replication [82].

2.4 Post-translational modifications of histones

A variety of post-translational covalent modifications for histones are known that include acetylation, methylation, phosphorylation, ubiquitylation, sumoylation and ADP-ribosylation. Even though most of these modifications have been already identified in the early years of chromatin research (reviewed in [1]) their impact on chromatin features has only been appreciated over the last years, leading to the “histone code“ hypothesis [83,84]. It proposes that different histone modifications act either synergistically, complementary or antagonistically as signals for regulatory events. For histone acetylation and histone methylation in particular numerous effects on chromatin mediated activities have been reported, and only these two modifications will be described in more detail here.

Histone acetylation is catalyzed by proteins containing a histone acetyltransferase (HAT) domain and affects both chromatin conformation and dynamics on the level of single nucleosomes as well as that of the higher order chromatin structure as reviewed recently [85]. HATs appear often in large, multiprotein assemblies such as SAGA for the GCN5 acetyltransferase [86]. Histone acetylation of certain residues in H3 and H4 correlates with transcriptional activation or DNA replication (H4K5 and K12) and is absent from heterochromatic structures [87,88]. Inhibition of the antagonistically acting histone deacetylases (HDACs) with compounds like trichostatin A leads to changes in the higher-order chromatin structure and the chromatin accessibility and points at the importance of this epigenetic mark in chromatin structure regulation [89,90]. Since acetylation of histone tails neutralizes the positive lysine charge it has been proposed that the electrostatic interactions of the tail with the DNA are weakened by acetylation, leading to destabilization of the nucleosome and respective higher-order structures. Thus, in addition to act as a recruitment (or eviction) signal to extrinsic factors, acetylation might have a direct structural impact on nucleosomes. In agreement with this view *in vitro* studies show that acetylation causes a decrease in folding of 30 nm structures, and promotes transcription [91-94].

Histone methylation can take place at either lysine or arginine residues [95,96]. Lysine methylation of histones involves mono-, di- and trimethylation of the lysine ϵ -nitrogen and is conferred by histone lysine methyltransferases (KHMTase) [95]. One class of KHMT is characterized by the presence of a SET-domain that was found in the *Drosophila* Su(var)3-9, enhancer of zeste (E(z)) and trithorax proteins. Well described residues for histone methylation at lysines are H3 K9, K4 and K79. The effect of histone methylation on the chromatin state is critically dependent on the residue modified and the number of methyl-

groups attached (for review see [97]). (Tri-)methylation of H3K4 for instance is an euchromatin marker. It is set by complexes containing the MLL (mixed lineage leukaemia) protein in humans and the SET1 protein in yeast. The recruitment of the complexes takes place via elongating factors associated with the transcribing RNA polymerase II, such as the Paf1 (polymerase II associated factor 1) complex in yeast. In contrast H3K9 methylation marks a step in a cascade of events that are necessary in the establishment and spreading of heterochromatin [98]. In addition to lysine modification, histone arginine methylation by protein arginine methyltransferases (PRMTs) has also been identified as an important epigenetic signal [96]. In mammals, a PRMT1- and CARM1-catalyzed modification to an asymmetric dimethyl-arginine histone has been associated with gene activation while a repressive effect has been reported for the symmetric dimethyl-arginine modification by PRMT5. Histone methylation has been long thought to be removable only by histone eviction. However, the recent discovery of a series of histone demethylases demonstrates the dynamic nature of this modification [99].

3. Structure of histone-DNA complexes

3.1 Nucleosome

The nucleosome is a roughly disc-shaped complex, in which 147 bp of the DNA are wrapped in 1.67 turns around the histone octamer (Fig. 3). Whereas each copy of the four core histones contacts the DNA in the nucleosome, only H3 and H2A interact with the other homotypic histone [5]. The N-termini of the core histones appear conformationally variable, which holds also true for the C-terminus of histone H2A. The highly cationic tails are the main targets of the post-transcriptional modifications described above [100-102] and serve as recognition motif for chromatin binding proteins such as HMGN-1, SIR 3-4 and others. They constitute important regions for interaction with chromatin remodeling factors [103,104], and play a vital role in the higher-order assembly of chromatin via inter-nucleosomal tail-tail interactions [105-107]. It is noted that the histone tails are not the only regions of interaction with additional protein factors. Recent studies demonstrate the recognition of histone-fold regions in the nucleosome by chromatin-associating factors and covalent modifications that lie on the surface of the nucleosome core structure [108,109]. The DNA in the core nucleosomal structure interacts with the histone octamer in 14 contact regions that are distributed over the inward facing side of the DNA. These can be referenced

by their superhelical location (SHL) from -6.5 to 6.5 and appear periodically with a distance of one full turn of the DNA where the DNA minor groove is faced towards the nucleosomal inner surface (see Fig. 3 C,D). The SHL describes the distance of a DNA residue to the central nucleosomal base pair. Thus, an SHL of 1 is a location of one DNA turn from the dyad axis away. Of the 14 protein-DNA contacts in the nucleosome, 12 are in the inner, highly bent 121 bp of core DNA.

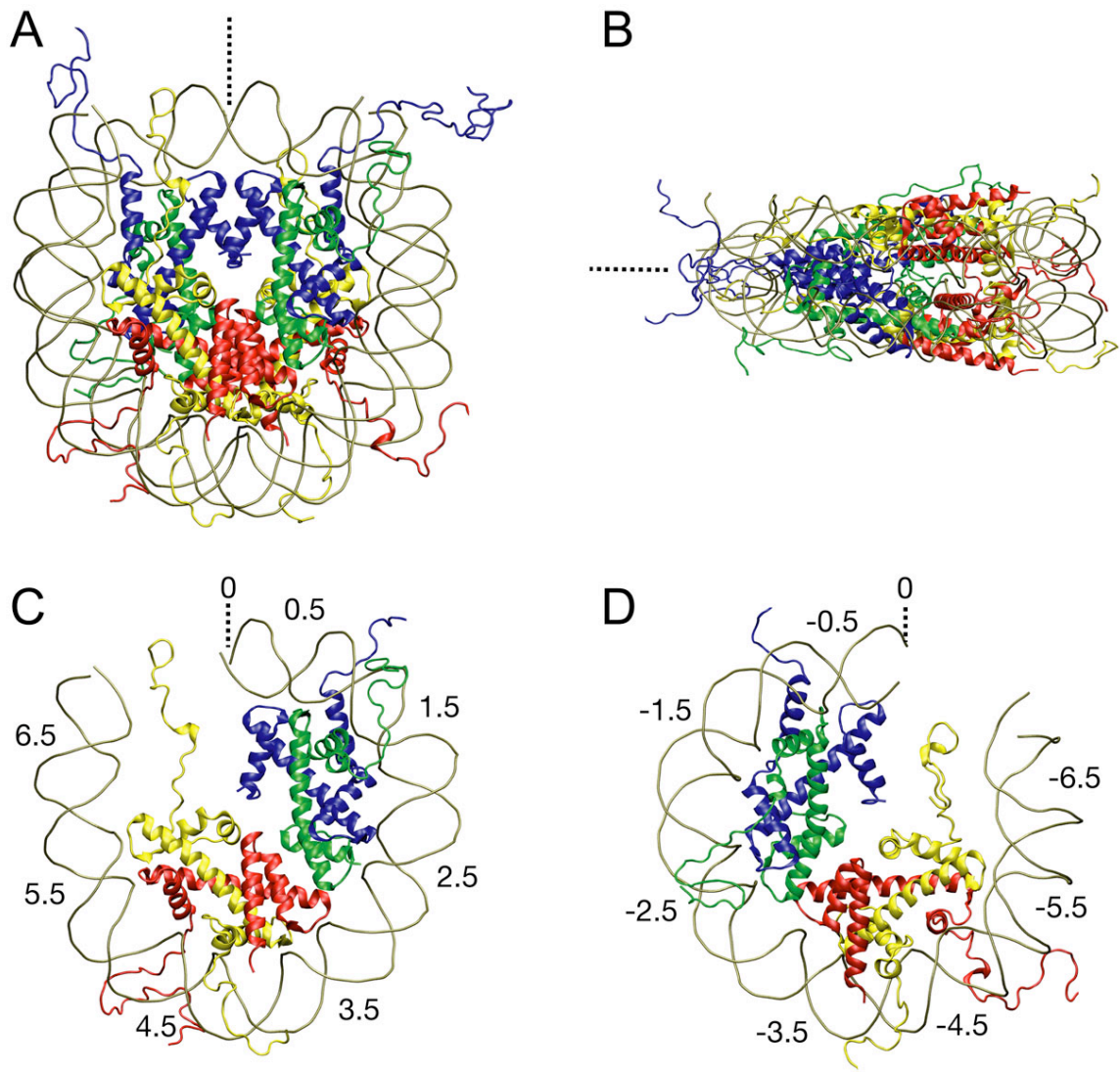


Figure 4. Molecular structure of the core nucleosome. The DNA is depicted as backbone line, the histones as ribbons. Histone proteins are colored blue for H3, green for H4, yellow for H2A and red for H2B. The dyad axis is depicted as broken line. (A) A top view of the nucleosome with vertical alignment of the dyad axis is shown. (B) The side view of the nucleosome is depicted. (C) depicts the upper half portion of the nucleosomal structure. (D) shows the corresponding lower half. The positions of superhelical locations are referenced by numbers. The structures were generated from the 147 bp X-ray nucleosome structure [6].

These interactions consist of water-mediated or direct hydrogen bonds between peptide groups or side chains with the phosphate-backbone of the DNA minor groove. In the crystal structure the histone tails partially locate in DNA minor grooves and follow them outwards of the core particle [6]. They are mostly unresolved, indicating a high structural flexibility in the crystals.

3.2 Chromatosome

The majority of nucleosomes is augmented by linker histones to a complex referred to as a chromatosome. Despite elaborate efforts, the exact structure of the linker histone bound to the nucleosome is still unknown. To characterize the DNA binding of the H1 group of proteins, a variety of DNA binding experiments have been carried out. It was demonstrated that linker histones bind cooperatively to linear double stranded DNA [110-112]. These studies also revealed that linker histones can form large complexes with relatively long DNA fragments hinting at more than one DNA binding site for the histone. Based on the crystal structure of the globular domain of histone H5 it was proposed to contain two DNA binding sites [32]. One of these has a winged helix DNA-binding motif (WH) for which a co-crystal structure has been reported [33]. The other putative DNA binding domain is a loop in the globular domain. It is less conserved and comprises a stretch of basic amino acids at the opposite surface of the globular domain. Gel analysis with four way junction DNA and directed mutations in the DNA binding domains supported the idea of two binding regions in the globular domain of H5 [113-115]. In addition, the C-terminal domain of H1 (CD) also serves as a DNA binding module [116-118].

Various models have been proposed for the integration of H1 and/or its globular domain in the nucleosomal structure [34,119-122]. Three of these are shown for the globular domain (Fig. 5). It is apparent that the binding of linker histone directly affects the geometry of the DNA entering/exiting the nucleosome. Most likely this will translate into changes of the higher order chromatin structure upon binding of linker histone. It is conceivable that multiple positions can be adopted by linker histones, which could explain the divergent findings of several groups. *In vivo* the positively charged C-terminal domain is essential for binding to chromatin [123]. This binding seems to be mediated by charge based interactions and also relies on a repeating S/TPXK motif (X refers to any amino acid).

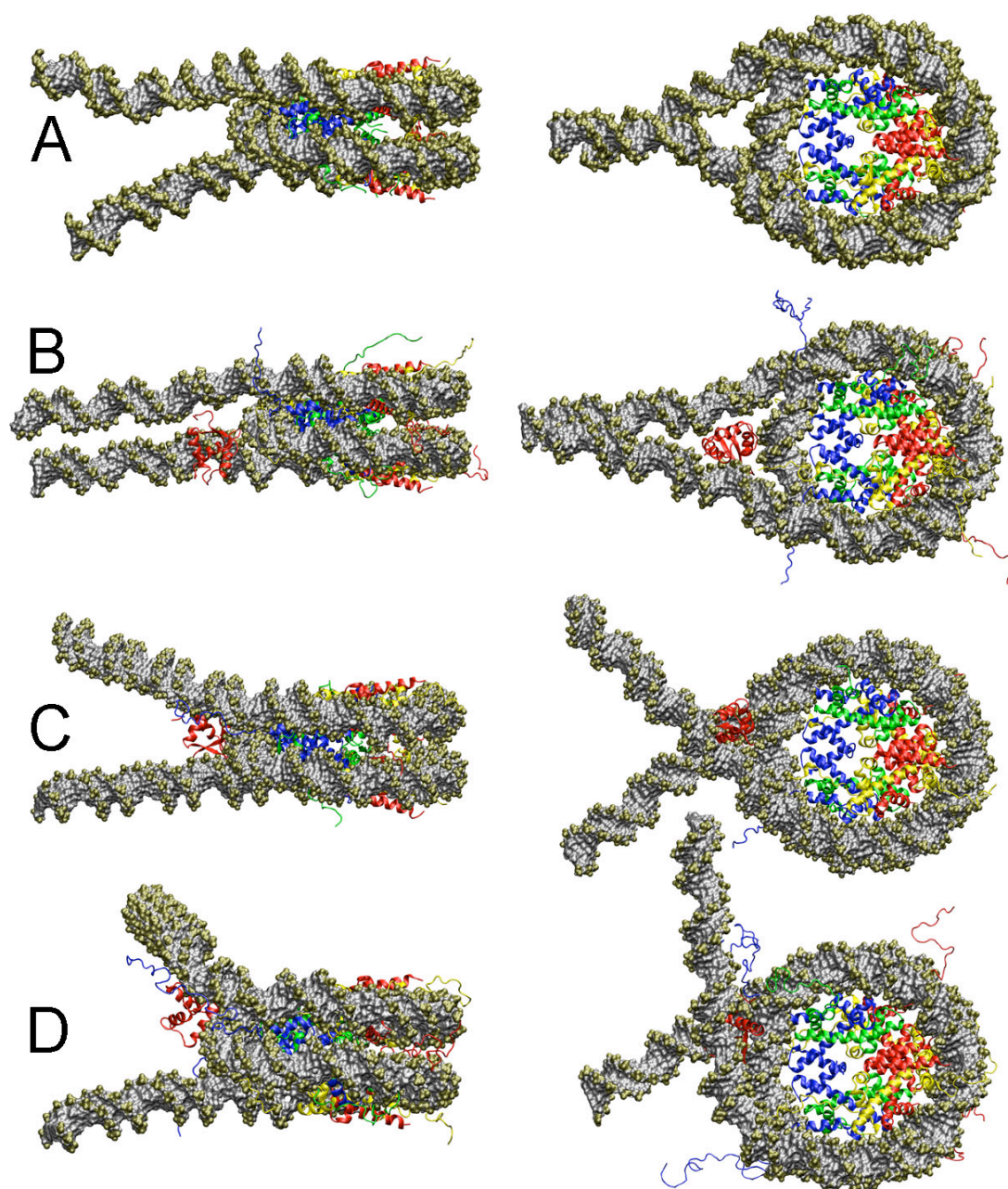


Figure 5. Model structures of the linker histone H1/H5 globular domain bound to the nucleosome. (A) For comparison a nucleosome with 199 bp of DNA was extracted from the tetranucleosome crystal structure [124]. It is noted that the interactions between the nucleosomes lead to some bending of the linker DNA. (B) Model for the globular domain interacting with the nucleosome derived from a chromosome structure with full length H1 [34] (C) Model from ref. [122] (D) Model from ref. [120].

4. Assembly of nucleosomes and chromatosomes

Under physiological salt concentrations the assembly of free histones into nucleosomes does not take place spontaneously, as the assembly process competes with the irreversible formation of unspecific histone-histone and DNA-histone aggregates. To prevent this possibly fatal interaction *in vivo*, histone expression and nucleosome assembly are tightly regulated and guided by additional factors, most notably the so called histone chaperones. For *in vitro* experiments nucleosomes can be reconstituted by salt gradient protocols. This widespread “salt”-reconstitution method circumvents aggregation that would occur by simply mixing histone octamer and DNA. It uses a gradual decrease of ionic strength via dialysis to assemble the particles in an ordered manner. During the dialysis, which typically begins at a 2 M monovalent salt concentration, the histone octamer dissociates [28]. The (H3·H4)₂ tetramer starts to interact with the DNA at an ionic strength of 1.2 to 1.0 M salt, forming a tetrasome particle. The H2A·H2B dimers specifically associate with this subnucleosomal assembly at 0.6-0.8 M salt, thereby completing the nucleosome structure [125,126]. At a concentration between 0.3-0.5 M salt the linker histones binds to the nucleosome. This assembly order is similar to the mediated by native factors, with the fundamental difference that the differences in ionic strength are likely to affect the energetics of the reaction as discussed below.

4.1 Chaperone guided nucleosome assembly

In vivo non DNA-bound histones are complexed with specific chaperones that have two functions. On the one hand they prevent non-specific DNA association leading to aggregation. On the other they ‘guide’ the specific nucleosomal assembly path. This is achieved by a thermodynamic balance between binding of the histones either to the chaperone, sub(nucleosomal) structures or non-specific association on DNA [49]. Histone chaperones include Nucleosome Assembly Protein 1 (NAP1) [127,128], ASF1 [129,130], Chromatin Assembly Factor 1 (CAF1) [131,132], N1/N2 [131,132], Nucleoplasmin [133-136], HIRA [137].

NAP1 has been characterized as the predominant chaperone for the H2A·H2B dimer *in vivo* in extracts from *Drosophila* embryos and the human HeLa tumor cell line [128,138]. *In vitro* the chaperone is capable of promoting complete nucleosome/chromatosome assembly as the sole carrier for all four core histones and the linker histone at physiological ionic strength

[49,128,139]. NAP1 co-purifies with HTZ1-Flag in yeast, pointing at its association with H2A.Z *in vivo* and a role in the generation of transcriptionally active chromatin by acting as histone donor for the SWR1 complex [51]. NAP1 was also shown to be involved in transcription control processes mediated by p300/CREB [140-143]. Besides its interaction with the H2A-H2B dimer, NAP1 functions as a linker chaperone in *Xenopus* oocytes [144] and can also fulfil this role *in vitro* [145]. On isolated chromatin fibers the chaperone regulates the H1 content in a concentration-dependent manner [146].

ASF1 is the major H3-H4 chaperone in the cell and forms a heterotrimeric complex of one ASF1 monomer together with one H3-H4 dimer [147,148]. It associates with CAF1 and HIRA and mediates the H3-H4 interaction of these chaperones [78,149,150]. The heterotrimeric CAF1 interacts directly via its largest subunit with PCNA (proliferating cell nuclear antigen, a component of the DNA polymerase η machinery) so that it is targeted to the replication fork. Via its interaction with ASF1 it serves in the assembly of (H3-H4)₂ tetrasomes on the newly replicated template [151]. In human cells, CAF1 is essential for nascent chromatin assembly and efficient S-phase progression [152]. In contrast the HIRA-ASF1 complex mediates the deposition of the variant H3.3 histone in a replication independent manner [78,137]. ASF1 is thought to function also as chromatin disassembly factor in yeast, as suggested by *asf1* mutant cells, which show decreased accessibility to micrococcal nuclease and DNase I [153,154]. Finally, ASF1 is involved in the assembly of silent chromatin [155], as well as in the dis- and reassembly of nucleosomes during RNA polymerase II transcription [156].

4.2 Chromatin remodeling complexes

Histone chaperones deposit nucleosomes in a rather random, unordered orientation to each other [128]. Even though the folding of a chromatin fiber is energetically favourable, with an estimated benefit of 0.8 to 2 kcal mol⁻¹ per internucleosomal contact [157,158], this structure will not be formed spontaneously due to randomly positioned nucleosomes with variable internucleosomal spacing. This points at the need for an ordered “primary structure” for higher order folding of the nucleosome chain, in which a regular spacing of nucleosomes on the DNA exists. Although nucleosomes show some spontaneous thermal mobility along the DNA, this process appears not to be sufficient for self folding of chromatin fibers [159]. Hence, albeit the 30 nm structure is energetically favourable, energy has to be spend for translocating nucleosomes along the DNA to potentiate the formation of a chromatin fiber. This process is conducted by the so called chromatin remodeling complexes. *In vivo*, these

complexes establish a proper chromatin context for central nuclear processes such as replication, DNA repair and gene expression. They move or evict nucleosomes to allow access to DNA for regulatory proteins, that otherwise would be impeded by their binding to the nucleosomal DNA [160], and confer the exchange of canonical histones for their variant counterparts as shown for H2A.Z. This broad range of activities is reflected by the severe phenotypes arising from knock-out of these complexes [161,162]. Chromatin remodeling complexes can be classified by their molecular composition, which evolves around the central DNA translocating and ATP consuming unit. Following this nomenclature, remodeling complexes are grouped into Snf2-, ISWI- Ino80-, Chd1-, Mi2- and other subfamilies (reviewed in [163-166]). Each group is characterized by the similarities in the ATPase, which all share a partial homology to the ATPase region of the Snf2 protein from yeast [167-169]. Each remodeling complex contains additional domains such as SLIDE, SANT and bromodomains, which confer specialized function.

The molecular mechanism of nucleosome movement is still under discussion. One model, referred to as the "twist model", postulates the propagation of a twist-defect along the histone-DNA surface. This leads to a base pair by base pair motion of the nucleosome along the DNA analogous to a screw nut progressing on a thread [170]. For translocation of the nucleosome a twist force has to be exerted on the DNA by the remodeling complex. This main aspect of the model has been challenged by various experimental findings. Most importantly, the incorporation of DNA nicks, gaps or bulky obstacles does not stop the activity of various remodeling complexes although these modifications would inhibit the transmission of a twist tension along the DNA [171,172]. Furthermore, the minimal step-size detected in reactions with ISWI and SWI/SNF class remodelers ranges from ~ 9-11 up to 50 bp, in contrast to the proposed one base pair step size [172-174]. An alternative model referred to as "loop-recapture" or "bulge-recapture" mechanism proposes the partial detachment of DNA on the entry/exit site of the nucleosomal surface. Additional DNA is pressed into the nucleosome, resulting in the formation of a DNA bulge, which migrates over the nucleosomal surface and leads to the translocation of the histone octamer. This model is supported by direct evidence for the formation of the looped intermediate by the ACF remodeling complex in DNA accessibility experiments [172]. One intriguing point in the mechanisms of chromatin remodeling complexes is the positioning specificity they provide for the nucleosome. Various results point at a translocation of nucleosomes by these complexes that is specific for the employed remodeler and varies with its complex

composition [172,173]. Even more, the positioning of single nucleosomes achieved by purified assembly extracts diverges from that seen with salt reconstitution in some systems despite of prolonged temperature shifts [175]. Hence, the *in vivo* positioning of nucleosomes does not necessarily reflect the thermodynamically optimal distribution for an isolated histone-octamer/DNA system.

5. Stability and dynamics of nucleosomes

5.1 Accessibility of nucleosomal DNA

Given the dynamics and the impact of local chromatin structure on a variety of nuclear processes, the stability of nucleosomes at a given DNA location has attracted longstanding research interest. In this context two of the key questions are how the stability of nucleosomes is determined by the bound DNA sequence, and by which means it can be modulated by additional factors and processes to allow interaction of DNA-binding factors with the nucleosomal DNA [176]. This is a critical determinant in the activity of numerous proteins as the highly distorted, partially buried nucleosomal DNA can not be accessed readily. This barrier can be broken by histone eviction or by sliding of nucleosomes via ATP-dependent mechanisms so that the target site is in the nucleosome linker region. In the absence of additional energy-dependent mechanisms nucleosomal assemblies show some spontaneous albeit transient unwrapping of their DNA in a process referred to as “breathing” *in vitro* [177-179]. Unwrapping appears pronounced for the DNA at the entry and exit sites of the nucleosome and decreases in probability towards the dyad axis. This agrees with fluorescence resonance energy transfer (FRET) data showing that the unwrapping proceeds from the ends of nucleosomal DNA to internal sites [180]. A quantitative analysis in single molecule measurements demonstrates that mononucleosomes on an average exist for 250 ms in a compacted state and dwell for 10-50 ms in an unwrapped state [180]. The ability for spontaneous unwrapping might be impeded by internucleosomal contacts, since the accessibility of histones is reduced in a fiber context as compared to mononucleosomes [146].

In the following the basis for specific DNA recognition by the nucleosome is discussed. It might be argued that the modulation of nucleosome stability (and positioning) by chromatin remodeling complexes and epigenetic modifications are the main determinants of chromatin

structure *in vivo*. However, recent evidence demonstrates that a correlation between *in vivo* positioning of nucleosomes and the *in vitro* binding in the absence of extrinsic factors exists, outlining the importance of DNA sequence on nucleosome positioning [181,182]. The high-resolution X-ray nucleosome structure provides detailed insight into the interaction between the DNA and the histone octamer. The previously mentioned 14 DNA-protein contacts in the nucleosome structure appear to contribute simultaneously to the recognition of specific DNA sequences. Base specific contacts between the protein content and the DNA are scarce [6]. In fact, the preference for DNA sequences is rather guided by geometric parameters, which allow a close association of the DNA with the histones at the contact points. This leads to a replacement of water-molecules with direct interactions between the nucleic acid and the protein, which is an entropically favorable energy contribution [6]. Given the distortion of the DNA at the interaction sites with the histones, it appears little surprising that mainly flexible AA, TA or TT tracts are positioned at these sites [181-186]. It has to be noted that this sequence specific bendability of DNA could provide an indirect “read out” of DNA sequence features by the histone octamer complex although no sequence specific protein-DNA contacts are made. In agreement with this view, high-affinity nucleosome binding sequences were shown to be more flexible than bulk DNAs [187].

5.2 DNA sequence specificity of nucleosome binding

The experimental assessment of thermodynamic and kinetic parameters for the nucleosome is far more challenging than for other nucleic acid binding components due to the multi-step assembly path and kinetic competition with aggregation. The relative affinity estimates for the DNA sequences tested up to date for nucleosome binding cover a range of $\Delta\Delta G = -4.1$ kcal mol⁻¹ between the strongest and weakest known artificial octamer binding sites [188]. Low affinity DNA binding is observed with artificial repetitive sequences like poly dG·poly dC or poly dA·poly dT [1] and repeats of TGGA [189]. The differences between high-affinity and bulk binding sites for the histone octamer found *in vivo* is relatively small and a value of -2.4 kcal mol⁻¹ has been reported [188]. In comparison, significant higher affinity differences between specific and unspecific DNA binding have been measured for other DNA-binding proteins with $\Delta\Delta G$ s between -5 to -9 kcal mol⁻¹ [190]. This suggests a low DNA sequence specificity of the histone octamer. In general, nucleosomes form readily with every natural DNA of sufficient length, consistent with their role as a universal compaction factor of eukaryotic DNA.

The above mentioned differences in the affinity of histones to certain DNA sequences have been predominantly determined *in vitro* by the “competitive reconstitution” assay. Originally, the underlying protocol was developed to facilitate effective incorporation of radioactive labels into mononucleosomes [191]. It uses a gradual decrease of ionic strength via dialysis to assemble the particles in an ordered way that has been described above. To measure affinity differences between two sequences, the reconstitution is carried out in the presence of an excess of an unspecific DNA, yielding a distribution between the two DNAs, which reflects their relative affinity during the assembly process. The approach allowed the screening for (artificial) high-affinity sequences [192]. Even on the strongest natural positioning sequences, like the 5S rDNA, salt-reconstituted nucleosomes distribute along a variety of positions, often in 10 bp steps [193]. This interferes with the generation of evenly spaced nucleosome arrays *in vitro*. Some artificial sequences (most notably the “Widom 601” sequence) have a single positioning site, and thus have been successfully used for the *in vitro* generation of evenly spaced oligonucleosomes in the absence of chromatin remodeling factors [194,195]. However, the results of the “competitive reconstitution” analysis have been subjected to some criticism [196]. Most importantly, the recognition of the DNA sequence takes place at high salt concentrations, where only some interactions between the histone H3·H4 tetramer and the DNA exist. At this stage of the assembly process “at near dissociating conditions” an equilibrium between the H3·H4 tetramer and different DNA sequences exists [196]. At lower salt concentrations a significant exchange of histones between the competing DNAs does not take place and affinity differences that arise only at physiological conditions are not reflected. Notably, the contribution of the two (H2A·H2B) dimers to nucleosome stability is neglected as they join the subnucleosomal structure at an ionic strength, at which the tetrasome is already firmly positioned. This is demonstrated by the observation that a similar relative binding energy is derived for a (H3·H4)₂ tetramer on 71 bp DNA, as for the complete nucleosome on the corresponding total 147 bp sequence [197]. The results seem to be also dependent on temperature, histone/DNA ratio and competing DNA sequence, which cannot be easily explained [198].

5.3 Thermodynamic and kinetic parameters for nucleosome formation under physiological conditions

One approach to determine the nucleosome free energies at physiological ionic strength is a stepwise dilution of nucleosomes down to concentrations at which the histone octamer dissociates from the DNA. Assuming an equilibrium between the complete nucleosome and the DNA/subnucleosomal species, an apparent dissociation constant or free energy can be determined [1,183,199-201]. From the results of Cotton and Hamakalo a K_d value for bulk

histones of ~ 3 nM ($\Delta G = -11.5$ kcal mol $^{-1}$) in a buffer supplemented with 0.15 M NaCl was derived [199]. A related approach was also used to study the nucleosome positioning sequence from the 5S rDNA, which has been used in a large number of *in vitro* studies of nucleosomes. The fraction of bound DNA was plotted against the nucleosome concentration and the data points were fitted to an equilibrium binding model to obtain $K_d = 0.2$ nM ($\Delta G = -13.2$ kcal mol $^{-1}$) for this DNA sequence in the presence of 0.15 M NaCl [201]. The salt dependence of the free energy in these experiments was approximately linear with the log of the ionic strength I and corresponds to $\Delta\Delta G/\Delta\log(I) = 1.2$ kcal mol $^{-1}$ [199,201]. Even though the approach appears straightforward, it also has its caveats. Nucleosome assembly at physiological salt concentrations competes kinetically with the formation of non-specific aggregates, which cannot mature into nucleosomes [202,203]. Thus, once the nucleosome dissociates, no true equilibrium is reached as shown in latter experiments [183].

In an alternative approach the thermodynamics and kinetics of the nucleosome (dis)assembly reaction on the 5S rDNA sequence were analyzed under equilibrium conditions at approximately physiological ionic strength (150 mM KCl, 2 mM MgCl $_2$) in the presence of stoichiometric amounts of the histone chaperone NAP1 [49]. Including the histone chaperone in the reactions prevents the irreversible formation of non-specific histone-histone and/or histone-DNA aggregates and ensures that the assembly process is reversible. The results from this type of analysis are summarized in Fig. 6 and Table 3. The specific formation of the nucleosome/chromatosome complex can be explained simply on the basis of thermodynamic differences in the interactions between chaperone, histones and DNA. In the case of NAP1 its affinity towards H3·H4 appears to be around that of linear DNA, thus causing a transfer of (H3·H4) onto DNA. In contrast, the relative affinity of H2A·H2B towards the chaperone must be above that of the H2A·H2B to DNA interaction, preventing release of H2A·H2B onto free DNA. Once a tetrasome structure is formed, it provides a higher affinity binding platform for H2A·H2B dimers so that the hexasome and nucleosome particles form. The latter structure is the preferred binding site for association with the linker histone so that the complete chromatosome complex assembles readily in a specific manner. For the multi-step reaction depicted in Fig. 6 the term “stability” of the nucleosome needs to be defined more precisely. Here, we refer to the final step in nucleosome formation, namely the binding of a second H2A·H2B dimer to the hexasome (Eq. 10 and Eq. 18). If the stability of the nucleosome is defined in terms of the fraction of histone free DNA an additional energy term for the dissociation of subnucleosomal species (hexasome, tetrasome and disome) from the DNA needs to be considered. In the energetics of the reaction shown in Fig. 6A the contribution of the interaction between NAP1 and the histone proteins is included.

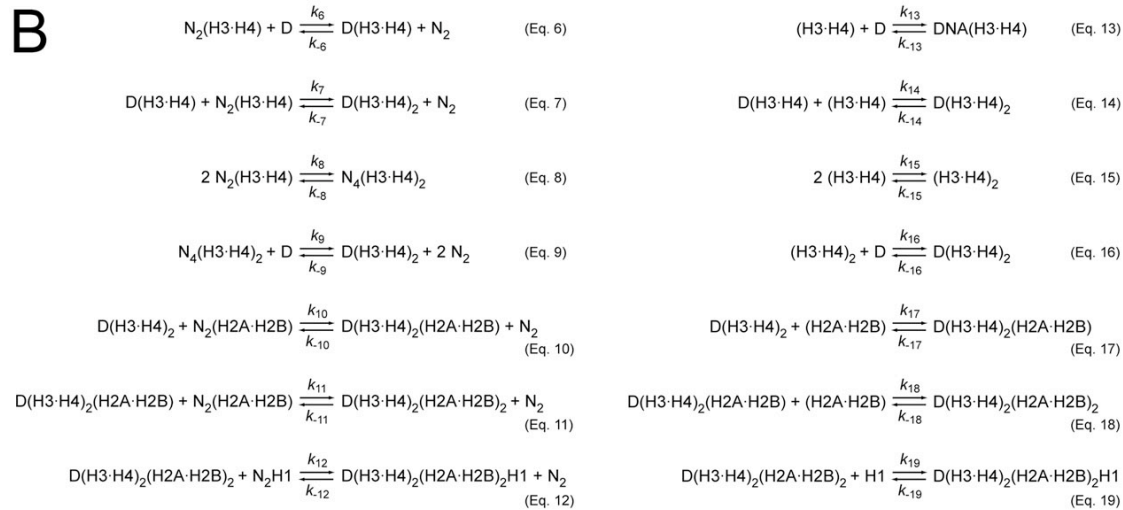
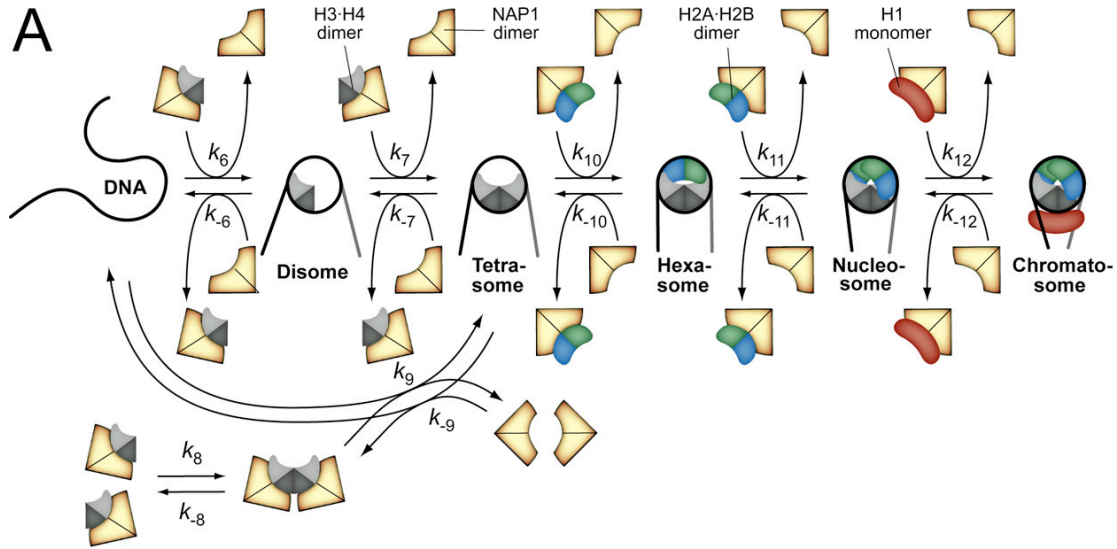


Figure 6. Mechanism of stepwise nucleosome assembly mediated by histone chaperone NAP1 [49]. The reaction is depicted as a process consisting of a series of reversible reactions with the corresponding forward and backward rate constants k . A schematic representation of the assembly (A) and the reactions (B) with NAP1 (Eq. 6 - 12, left panels) and without NAP1 (Eq. 13 - 19, right panel) are shown. D is the DNA fragment and N_2 is the NAP1 dimer. Under the conditions of the experiments referred to here NAP1 is predominantly present as a dimer and binds as a dimer to a H3-H4 dimer, a H2A-H2B dimer or a H1 monomer [49,146,204]. Two $N_2(\text{H3-H4})$ complexes react consecutively with the DNA to form a tetrasome particle and two free NAP1 dimers. The tetrasome particle reacts with a $NAP1_2(\text{H2A-H2B})$ to form a hexasome particle. This complex is augmented with a second H2A-H2B dimer from another $N_2(\text{H2A-H2B})$ complex, resulting in a complete nucleosome and release of N_2 . Finally, the linker histone H1 is added to the nucleosome.

To a first approximation the binding affinity of the NAP1 dimer is the same for one H3-H4 dimer, one H2A-H2B dimer or one H1 monomer, and a value of $\Delta G = -10.2 \pm 0.4 \text{ kcal mol}^{-1}$ ($K_D = 30 \pm 20 \text{ nM}$) was determined (Mazurkiewicz & Rippe, unpublished). This is in

agreement with a previously reported value of $K_d = 20$ nM for the NAP1 histone interaction [205]. The NAP1 contribution can be subtracted to derive stability values in the absence of a histone chaperone according to the Eqs. 13 to 19 in Fig. 6B. For example, the ΔG for the binding of the second (H2A·H2B) dimer to form a nucleosomes from a hexasome is -1.5 ± 0.2 kcal mol⁻¹ with NAP1 and corresponds to $\Delta G = -11.7 \pm 0.5$ kcal mol⁻¹ or $K_d = 3$ nM in the absence of histone chaperone (Table 3). This value is very similar to that calculated as the average for site specific DNA binding of proteins of 11.7 ± 1.6 kcal mol⁻¹ under comparable conditions [190]. Thus, the nucleosome is a relatively stable protein-DNA entity. The presence of histone chaperones leads to a considerable increase of the dynamics of the complex and facilitates the exchange of histones. This is particularly evident from a comparison of the calculated half life of (sub)nucleosomal particles. While a value of $t_{1/2} = 11.5$ min is calculated for the nucleosomes in the presence of 2 μ M free NAP1 dimer, it is estimated that the dissociation of one H2A·H2B dimer from a nucleosome would occur on the hour time scale in the absence of a histone chaperone (Table 3). All data in Table 3 refer to the assembly/dissociation of an isolated nucleosome. As described above, nucleosomes with a regular spacing will assemble into a chromatin fiber structure in which the nucleosome is stabilized by an estimated 0.8 to 2 kcal mol⁻¹ [157,158]. This additional stabilization could easily translate into a tenfold slower off rate for the dissociation of a (H2A·H2B) dimer from the nucleosome and a corresponding increase of its residence time. Interestingly, *in vivo* half times of 130 min for the bulk of the histone H2B dimer were measured, while a 3% fraction of H2B is being exchanged within minutes. This rapidly exchanging fraction of H2B disappeared in the presence of the RNA polymerase II inhibitor 5,6-dichloro-1-beta-d-ribofuranosylbenzimidazole and has been assigned to transcriptionally active regions with a more open chromatin organization [206]. This relation is also supported by the observation that transcription elongation is facilitated by factors that alter nucleosomes in order to allow RNA polymerase to proceed through chromatin. In particular, the movement of RNA polymerases along the template involves extracting one H2A·H2B dimer from the nucleosome so that a hexasome complex appears as an intermediate of transcription [207-211]. Thus, the higher order organization of the nucleosome chain appears to provide a mechanism to modulate the stability of histone-DNA complexes and to facilitate or impede transcription.

Table 3. Thermodynamic and kinetic parameters for nucleosome assembly

NAP1 mediated nucleosome assembly ^a					
	Disome (Eq. 6)	Tetrasome (Eq. 7)	Hexasome (Eq. 10)	Nucleosome (Eq. 11)	Chromatosome (Eq. 12)
ΔG (kcal mol ⁻¹)	3.7 ± 0.9	- 2.4 ± 0.6	-4.1 ± 1.8	-1.5 ± 0.2	-0.6 ± 0.2
K_d	(5.4 ± 2.2)·10 ²	(1.8 ± 0.8)·10 ⁻²	(1.1 ± 0.8)·10 ⁻³	(8.5 ± 1.8)·10 ⁻²	(3.4 ± 2.3)·10 ⁻¹
k_{on} (M ⁻¹ s ⁻¹)	(2.5 ± 0.7)·10 ⁴	(4.7 ± 1.4)·10 ⁵	(4.1 ± 2.5)·10 ⁵	(5.9 ± 0.5)·10 ³	(7.7 ± 3.7)·10 ³
k_{off} (M ⁻¹ s ⁻¹)	(1.4 ± 0.4)·10 ⁷	(8.7 ± 2.6)·10 ³	(4.4 ± 2.0)·10 ²	(5.0 ± 1.0)·10 ²	(2.6 ± 1.2)·10 ³
$t_{1/2}$ (s) ^b	0.03 ± 0.008	40 ± 12	790 ± 360	690 ± 140	130 ± 60

Predicted parameters for nucleosome assembly without chaperones ^c					
	Disome (Eq. 13)	Tetrasome (Eq. 14)	Hexasome (Eq. 17)	Nucleosome (Eq. 18)	Chromatosome (Eq. 19)
ΔG (kcal mol ⁻¹)	-6.5 ± 1.1	-12.6 ± 0.8	-14.3 ± 1.9	-11.7 ± 0.5	-10.9 ± 0.5
K_d (M)	(1.7 ± 1.1) ·10 ⁻⁵	(5.8 ± 3.8)·10 ⁻¹⁰	(3.4 ± 2.6)·10 ⁻¹¹	(2.7 ± 1.4) ·10 ⁻⁹	(1.1 ± 0.7) ·10 ⁻⁸
k_{off} (s ⁻¹) ^d	(4.3 ± 3.0) ·10 ⁻¹	(2.7 ± 1.9) ·10 ⁻⁴	(1.4 ± 1.3) ·10 ⁻⁵	(1.6 ± 0.8) ·10 ⁻⁵	(8.1 ± 6.4) ·10 ⁻⁵
$t_{1/2}$ (h)	(5 ± 3) ·10 ⁻⁴	0.7 ± 0.5	14 ± 13	12 ± 6	2.4 ± 1.8

Parameter refer to the reactions depicted in Fig. 6 with a 207 base pair containing the 5 S rDNA sequence in a buffer supplemented with 150 mM KCl and 2 mM MgCl₂ and a temperature of 25 °C.

^a Kinetic on and off rates were determined with stoichiometric amounts of histone chaperone NAP1, from which $K_d = k_{off}/k_{on}$ and $\Delta G = RT \ln(K_d)$ at 25 °C was derived [49].

^b The half-time for the decay of the complex was calculated according to $t_{1/2} = \ln(2)/k'_{off}$ with k'_{off} being the apparent off rate in units of s⁻¹ that includes a constant concentration of 2 μM of free NAP1 dimer. To our knowledge no values for the concentration of free histone chaperones in the cell have been reported. However, some estimates can be made with respect to the intranuclear concentrations of core histones and chaperones. In experiments with autofluorescent histone that constituted only about 5% of the endogenous histone pool, a fraction of 4-11% mobile histones has been measured in HeLa cells [89,206,212]. With an average nucleosome concentration of around 140 μM in HeLa cells

during interphase [212] this would correspond to the equivalent of around 10 μM histone octamer not incorporated into chromatin. Accordingly, the total concentration of histone chaperones should be at least in the 10 μM range similar and sufficient to complex at least 1 μM free histone octamer as estimated previously for NAP1 [204].

^c The stability of histone-DNA complexes in the absence of histone chaperone was calculated with a free energy $\Delta G = -10.2 \pm 0.4 \text{ kcal mol}^{-1}$ for the interaction of a NAP1 dimer with one H3-H4 dimer, one H2A-H2B dimer or one H1 monomer. A value of $K_d = 30 \pm 20 \text{ nM}$ was determined for the interaction of a NAP1 dimer with a H2A-H2B dimer by fluorescence anisotropy measurements. In competitions experiments with H3-H4 dimer or H1 monomer a similar affinity to NAP1 was observed (Mazurkiewicz & Rippe, unpublished).

^d This parameter was calculated by assuming that the stability increase by $-10.2 \text{ kcal mol}^{-1}$ of the histone-DNA complex in the absence of chaperone NAP1 leads to a corresponding reduction of the dissociation rate constant as would be predicted by transition state theory [213,214].

For the binding affinity of the linker histone H1 to the nucleosome a free energy of $-10.9 \text{ kcal mol}^{-1}$ ($K_d = 11 \text{ nM}$) was calculated here for the interaction in the absence of histone chaperone (Table 3). This is consistent with a previously determined dissociation constant of $K_d = 2 \text{ nM}$ for the binding of one H1 to the nucleosome at a somewhat lower salt concentration of 50 mM NaCl, which is expected to lead to tighter binding of H1 [215]. *In vivo*, the linker histone H1 shows a rapid exchange on the second time scale between the chromatin bound and the free form in fluorescence recovery after photobleaching experiments with half times of 20 to 50 s for the recovery of the fluorescence [216,217]. From this type of experiments kinetic rate constants $k_{\text{on}} = 0.14 \text{ s}^{-1} \text{ M}^{-1}$ and $k_{\text{off}} = 0.0131 \text{ s}^{-1}$ ($K_d = 0.096 \text{ M}^{-1}$) have been derived [218]. The later off rate corresponds to $t_{1/2} \sim 50 \text{ s}$ for H1 binding to chromatin *in vivo*, which is similar to the value of $t_{1/2} = 130 \pm 60 \text{ s}$ estimated here for the *in vitro* assembly reaction with NAP1 (Table 3). This suggests that the rapid exchange of linker histone *in vivo* is maintained by its interaction with histone chaperones, as the dissociation rate in the absence of histone chaperones would be significantly reduced.

6. Higher-order chromatin structures

6.1 Assembly of chromatin fibers

Nucleosomal arrays, i. e. DNA fragments with multiple nucleosomes isolated from cells adopt a “beads on a string” conformation with regular spacing of nucleosomes when incubated at low salt concentrations. Adjacent nucleosomes are separated by a linker DNA

segment of 20-80 bp depending on species and cell type [1]. This yields a total nucleosomal repeat length (NRL) of 165-220 bp per nucleosome. Intriguingly, the distribution of spacer length is not random but follows a ~10 bp periodicity [219], which closely resembles a helical turn of DNA (10.4 bp). This points at the before mentioned sterical requirement of nucleosome arrangement in higher-order folding. Furthermore, calculations based mainly on data of recent knock-out studies demonstrate a linear relationship between the ratio of H1 per nucleosome and the NRL. The results show that the presence of the linker histone leads to a lengthening of the NRL by 37 bp [220]. At physiological salt concentration, longer fragments can reversibly fold into a fiber characterized by a diameter of approximately 30 nm if nucleosomes are bound to DNA with a regular spacing [221,222]. Accordingly, a minimal assembly system for chromatin fiber formation requires histone chaperones and chromatin remodeling complexes in addition to DNA and histones [223]. As discussed previously the fiber formation is likely to proceed via the mechanism depicted in Fig. 7 [49]. The reaction starts with the histone chaperones guided assembly of core, variant and linker histones with DNA into nucleosomes and chromatosomes, in which unspecific histone aggregation as well as the formation of DNA-(H2A-H2B) complexes that cannot mature into nucleosomes are prevented by the chaperone [144,224,225]. Initially, nucleosomes, chromatosomes and hexasomes are likely to be present simultaneously in rapid equilibrium to form the nascent chromatin fiber with an irregular spacing of these particles. Due to the activity of chromatin remodelers a regular spacing of nucleosomes is established so that the chain can fold into a chromatin fiber structure. In this structure the equilibrium between hexasomes and nucleosomes is shifted towards the nucleosome, as core histones are protected from extraction by histone chaperones through internucleosomal contacts.

Even though elaborate efforts have been made to solve the structure of the chromatin fiber, various models for the fiber geometry are still under current investigation. They can be classified into a continuous solenoidal wrapping of the nucleosomes chain (one-start helix) or two-start fibers with a more zigzag like shape. Furthermore, they differ with respect to the orientation of the nucleosome to the helix axis, the position of the linker histone, the degree of linker DNA bending and in their ability to accommodate different nucleosome repeat lengths [1]. It is also noted that an alternative model to that of a continuous fiber is the "superbead" model by Franke and coworkers, in which 8 nucleosomes (chicken and rat liver) to 48 nucleosomes (sea urchin sperm) associate into a globular particle [226].

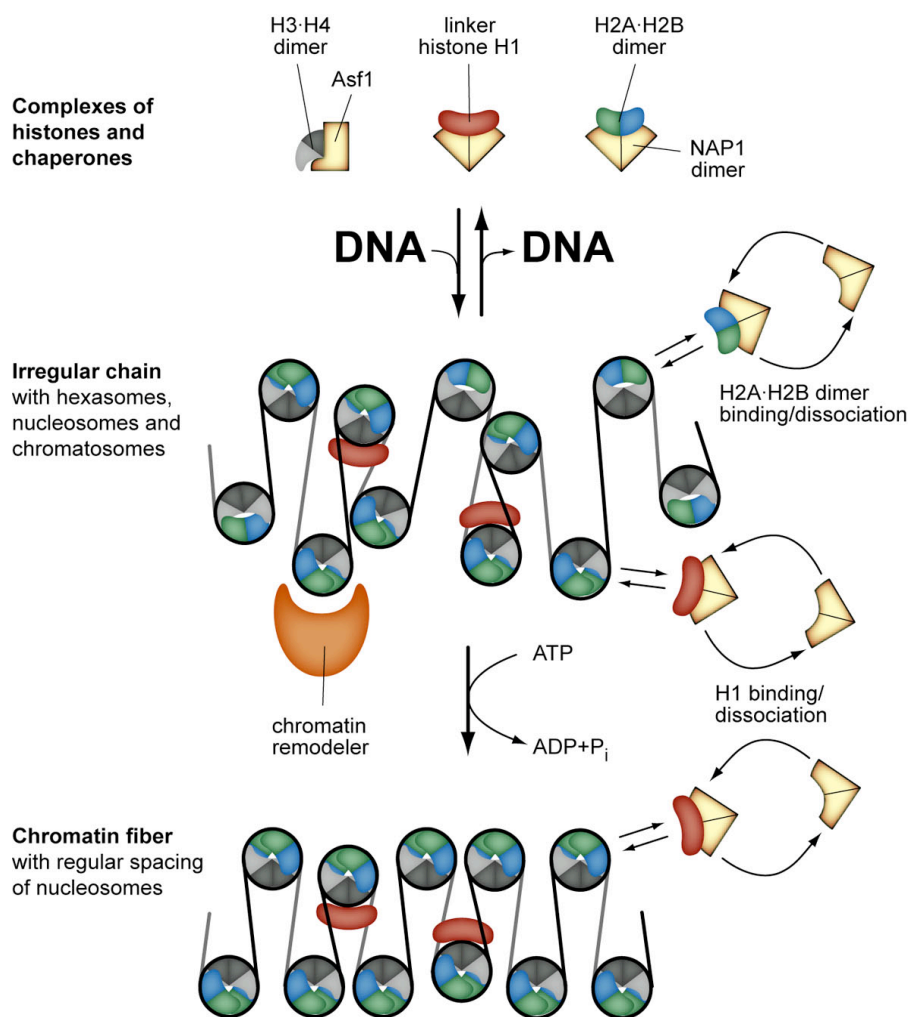


Figure 7. Mechanism for the assembly of a chromatin fiber. Histone chaperones like ASF1 for the (H3·H4) dimer and NAP1 for the (H2A·H2B) dimer deposit histones on the DNA to form an irregular chain with hexasomes, nucleosomes and chromatosomes. In this conformation the (H2A·H2B) dimer is in fast exchange between the free and the DNA bound state. This irregular chain is converted into a structure with regularly spaced nucleosomes by ATP dependant remodeling complexes. In this conformation a chromatin fiber is established, in which the nucleosomes are stabilized. Thus, H2A·H2B dimer dissociation is impaired and the hexasome state is depleted. In contrast, the chaperone mediated binding and dissociation of linker histone remains possible.

The “classical” solenoid model proposes an one-start organization of the fiber [35,227-229]. A critical feature of this model is that interactions occur between adjacent nucleosomes on the DNA. This requires bending of the intervening linker DNA, which could be facilitated by association with linker histone H1 [230]. However, linker DNA bending is energetically unfavorable and a number of findings argue in favor of straight linker DNA [231]. Two-start fiber models with straight linker DNA propose a conformation, in which neighboring

nucleosomes on the DNA are oriented on different sides of the fiber with their connecting linker DNA crossing the inner section so that internucleosome contacts are made between nucleosomes at positions i and $i + 2$ [232]. In these structure the fiber diameter would be expected to depend linearly on the length of the linker DNA [233]. One of this type of fibers structures is shown in Fig. 8. It is based on X-ray structures of a tetranucleosome complex, high-resolution electron microscopy work, cross-linking and *in vivo* mapping [124,194,234].

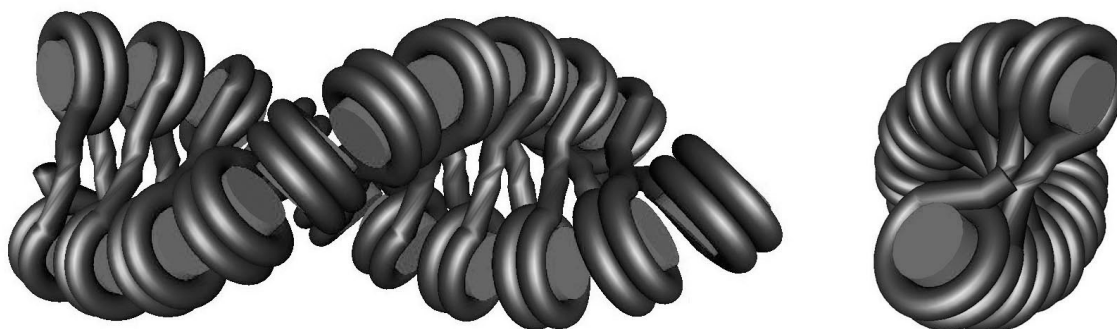


Figure 8. Two-start helix chromatin fiber as proposed by Schalch et al. [124]. The fiber model is based on the X-ray structure of a tetranucleosome that is extended into a fiber.

The tetranucleosome structure used as the building block for the fiber has been determined with a relatively short 167 bp repeat length in the absence of linker histones [124]. It provides the first high resolution structure for interactions between nucleosomes in higher-order folding of a nucleosomes chain. Recently, an alternative chromatin fiber model has been suggested on the basis of electron microscopy studies, in which a series of nucleosome arrays was imaged with repeat lengths of 177, 187, 197, 207, 217, 227 and 237 bp and one linker histone per nucleosome [195]. The analysis by Robertson et al. identified two distinct structural classes of fibers. One type of fiber adopted by nucleosome repeat lengths of 177–207 bp has a diameter of 33 - 34 nm and a nucleosome packing ratio of 11 nucleosomes per 11 nm. Longer repeat lengths of 217 - 237 bp associated into thicker fibers with a diameter of 43 nm and a mass density of 15 nucleosomes per 11 nm. From these results a 30 nm fiber structure was derived, in which nucleosomes from successive gyres interdigitate in a left-handed one-start helix with 5.4 nucleosomes per helical turn [195] similar to a previously proposed model [235].

6.2 Higher order folding of chromatin fibers

Several lines of evidence indicate that the chromatin fiber adopts a higher order folding that organizes the interphase chromosome into domains containing roughly 1 Mb of DNA [236,237]. Using high resolution light microscopy an apparent bead like structure of chromatin can be visualized, in which chromatin domains of this genomic size are more densely packed into an approximately spherical subcompartment structure with dimensions of 300 - 400 nm [238].

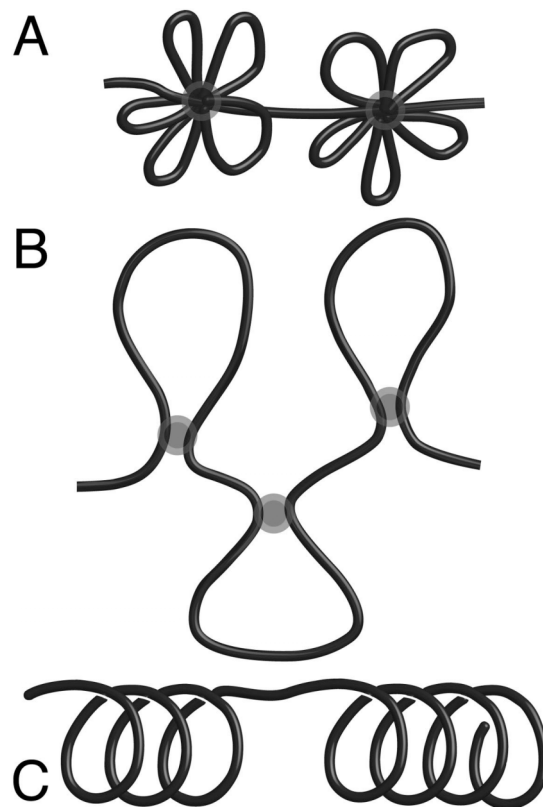


Figure 9. Hypothetical models for the folding of the chromatin fiber during interphase leading to the formation of 1 Mb chromatin domains. (A) the radial-loop model, (B) the random-walk/giant loop (RW/GL) model or (C) the chromonema model.

However, the resolution of the light microscope is not sufficient to identify the organization of the 30 nm chromatin fiber that leads to the formation of the ~1 Mb domains and different conformational states have been proposed (Fig. 9). In the radial-loop models the 30 nm fiber forms loops of roughly 100 kb size that are arranged into rosettes [239-242] (Fig. 9A). The random-walk/giant-loop (RW/GL) model (Fig. 9B) suggests the looping of large regions of chromatin (3 Mb) and tethering of these structures to a backbone like structure [243]. In the

chromonema model [244-246] the compaction of the 30 nm fiber is achieved by its folding into 60 - 80 nm structures that undergo additional folding to 100 - 130 nm chromonema fibers (Figure 9C).

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