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Minireview

Structure and dynamic properties of nucleosome core particles

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Abstract It is now widely recognized that the packaging of genomic DNA, together with core histones, linker histones, and other functional proteins into chromatin profoundly influences nuclear processes such as transcription, replication, DNA repair, and recombination. Whereas earlier structural studies portrayed nucleosomes (the basic repeating unit of chromatin) as monolithic and static macromolecular assemblies, we now know that they are highly dynamic and capable of extensive crosstalk with the cellular machinery. Histone variants have evolved to locally alter chromatin structure, whereas histone chaperones and other cellular factors promote histone exchange and chromatin fluidity. Both of these phenomena likely facilitate interconversion between different chromatin states that show varying degrees of transcriptional activity.

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1. Introduction

The genetic information of a single eukaryotic cell is stored in DNA molecules over two meters in length. In the nucleus, it is compacted to nearly one hundred thousandth of this dimension by a hierarchical scheme of folding with an equal mass of proteins, forming a nucleoprotein complex called chromatin [1] (Fig. 1). At the first level of organization, nearly two tight superhelical turns of DNA (147 base pairs) are wrapped around a disk-shaped protein assembly of eight histone molecules to form the nucleosome core particle (NCP) [2,3]. Long arrays of nucleosomes, connected by linker DNA of variable length, are further compacted in multiple higher organizational levels of unknown architecture [4]. Also critically involved in this process are the flexible histone tails, linker histone H1, a variety of non-histone proteins, polyamines and divalent metal ions (Fig. 1).

The accessibility of DNA that is sequestered in chromatin differs dramatically from that of linear protein-free DNA. This has fundamental implications for our understanding of all biological processes that use DNA as a substrate, such as transcription, replication, DNA repair, and recombination. The inter-conversion of the fluid chromatin structures that prevail in the interphase nucleus [5] from transcriptionally blocked to transcriptionally active states are likely to be tightly regulated by reversible modification of histones, of other associated proteins, and of DNA (reviewed in [6–8]). Additional mechanisms to control chromatin structure and thus DNA accessibility at several levels involve the targeted action of ATP-dependent chromatin remodeling factors (reviewed in [9]), and the introduction of histone variants. Histone variants are specialized core histones that replace major-type histones mostly via replication-independent assembly pathways. They exhibit specific spatial and temporal patterns, and their unique structural properties contribute to the formation of altered chromatin structures (reviewed in [10,11]). Here we provide a brief overview of advances in our understanding of nucleosome structures. We will further discuss recent findings that emphasize the dynamic aspect of these large macromolecular assemblies.

2. Lessons from 27 nucleosome structures

The recent years have brought much progress in our understanding of the structure of the nucleosome core particle (reviewed in [3]). The amount of structural information available from the 1.9 Å structure of the NCP, reconstituted from recombinant *Xenopus laevis* histones and a 'symmetric' 147 bp DNA fragment derived from human α-satellite DNA is unprecedented [12,13]. A comparison with the crystal structures of NCPs from chicken [14], yeast [15], Drosophila (Chakravarthy et al., unpublished results) and mouse (Chakravarthy et al., unpublished results) shows how most minor sequence changes are accommodated within this complex without significant structural changes. Importantly, sequence variations are not restricted to the surface of the NCP, but are also found buried at protein–protein interfaces (see, for example, [15]).

To investigate the contribution of several invariant key residues at a histone–DNA interface near the dyad we determined eleven NCP structures, each harboring an individual point mutation in either histone H3 or H4 (Fig. 2A). An examination of their structural and dynamic properties shows that single amino acid changes result in the loss of a very limited number of contacts with the DNA, leading to an increased propensity of these mutant nucleosomes to slide along the DNA, whereas the overall path of the DNA and protein is mostly unchanged [16] [17]. Thus, the disruption

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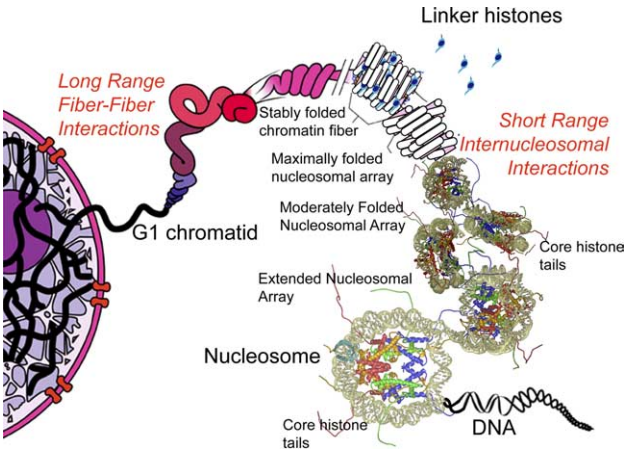

Fig. 1. Eukaryotic DNA is organized in a modular fashion. The multiple levels of DNA compaction are depicted in a schematic manner. Long arrays of nucleosomes (atomic structures are shown at lower levels of compaction, replaced later with schematic representations) are compacted via short-range and long-range interactions into fibers of unknown architecture.

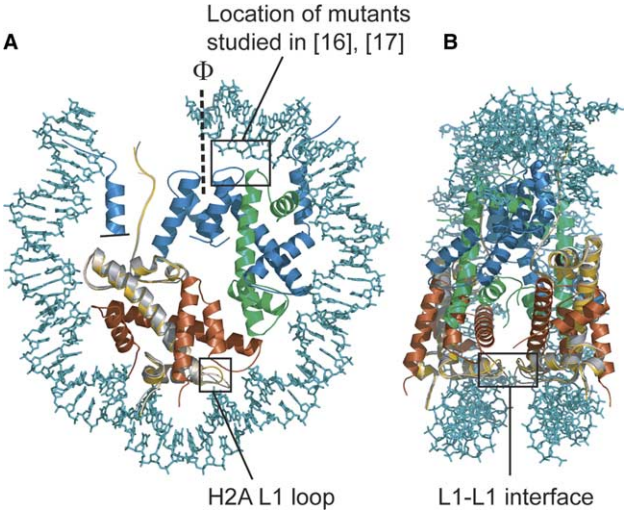

Fig. 2. Nucleosomes containing histone H2A variants differ mainly in the L1 loop. (A) One half of the nucleosome structure is shown, viewed down the superhelical axis. H3, H4, and H2B are shown in blue, green, and red, respectively; the DNA is shown in green. Major type H2A is shown in yellow, and the structure of macroH2A and H2A.Z are superimposed and shown in gray and wheat, respectively. Regions that are discussed in the text are indicated.

of only two to six of the ~120 direct histone–DNA interactions within the nucleosome has a pronounced effect on nucleosome mobility and stability, exemplifying an amazing degree of fine-tuning of the protein–DNA interfaces within the nucleosome. Most of the mutations studied in [16,17] have been shown to alleviate the effects of the inactivation of an ATP dependent chromatin remodeling factor in vivo [18]), suggesting that ATP is utilized to lower the energy barrier for nucleosome sliding.

The structures of NCPs in which major H2A has been replaced by the specialized histone variant H2A.Z [19] or with the histone-like domain of the variant macroH2A (Chakravarthy et al., unpublished results) reveals that even substantial sequence variations are accommodated in the nucleosome without major structural distortions. Sequence identity between these histone variants and major-type H2A is only around 60%. Given the extreme degree of conservation of histone amino acid sequences throughout evolution [20], this can be considered highly divergent. Together, our structural data suggest that the overall structure of the nucleosome is actually quite forgiving towards sequence changes. In hindsight our results are not altogether surprising, since the binding and supercoiling of DNA remains the fundamental function of the nucleosome above and beyond all other roles. One possible exception are nucleosomes containing the H2A variant H2A.Bbd, where a less efficient binding of the ends of the DNA (as demonstrated by fluorescence resonance energy transfer and micrococcal nuclease digestion) results in reduced stability [21,22]. However, the degree of structural deviation of such nucleosomes from canonical nucleosomes will only become obvious once the molecular structure is available.

One particular region in histone H2A, the L1-loop, seems to have been the consistent target for variability in the evolution of histone H2A not only from lower to higher eukaryotes but also of the numerous non-allelic variants. In particular, a four-amino acid stretch in this loop forms the L1L1 interface, which in addition to being the sole region of interaction between the two H2A–H2B dimers also appears to be holding together the two gyres of superhelical DNA in the nucleosome (Fig. 2B). It is therefore not far-fetched to imagine that subtle sequence variations in this region may render the nucleosome susceptible or resistant to key cellular processes such as transcription, while maintaining its overall structural integrity. The relative affinity between the L1-loops of different H2A variants and that of major-type H2A may be the governing factor in the regulation of the histone content/stoichiometry of nucleosomes. Other potentially significant sequence differences in H2A variants are seen on the charged surface of the variant nucleosomes and the unstructured histone tails [11]. Both these regions have been implicated in facilitating higher order structure formation [23]. Sequence differences may give rise to an alternative pattern of covalent modifications that may translate either directly to an altered level of chromatin compaction or to a recruitment platform for an alternative set of chromatin-associated non-histone proteins.

3. Distinct structural changes in the nucleosome upon ligand binding

Structural and functional studies of NCPs in complex with small, minor-groove DNA binding ligands (the pyrrole–imidazole polyamides; [24]) have demonstrated an additional aspect of nucleosome structure that may well relate to its in vivo function. It appears that nucleosomal DNA is quite accessible for recognition [25], despite the tight interaction between DNA and histones, and the dramatic deviation of the structure of nucleosomal DNA from canonical B-form DNA [13]. Nucleosomal DNA is also surprisingly malleable in that it is able to accommodate significant structural distortions imparted by DNA binding ligands without losing contact with the histone octamer [26,27]. Using X-ray crystallography and footprinting techniques, we showed that nucleosomal DNA exists in a dynamic equilibrium of multiple 'twist diffusion' intermediates in solution [28], further emphasizing the dy-

namic character of a structure that has until recently been perceived as a monolithic and static macromolecular assembly.

The pyrrole–imidazole polyamides used in the above study, although comparable to transcription factors in their binding specificity and affinity, fit snugly into the minor groove of DNA [26] and lack the 'bulk' of DNA binding proteins. The structural consequences of transcription factor binding on the structure of a mono-nucleosome have been investigated using fluorescence resonance energy transfer (FRET) and other techniques [29,30]. It was found that a target site that is buried due to the interaction with histones is made accessible by a dynamic partial unwrapping of the DNA. This holds true for two different transcription factors, namely Amt1 from the pathogenic yeast *Candida glabrata* [31], and the much-studied transcription factor LexA. The binding sites for these proteins that have very different DNA recognition modules are located near the dyad and near the end of the nucleosomal DNA, respectively [30,29]. Amt1 binding at the nucleosomal dyad is accompanied by the partial dissociation of the DNA ends from the histone octamer surface that appears to be necessary to free up the binding site and to generate room for the transcription factor. However, no dissociation or even subtle rearrangements of histone subunits is observed in this particular system [30]. Similarly, LexA binding towards the end of the DNA is made possible by a breathing of the DNA ends [29], in accordance with the 'site exposure model' proposed by the same laboratory [32], and references therein). This model postulates that the ends of the DNA are in rapid equilibrium between histone-bound and unbound state, and that the unbound state may be captured and stabilized by the binding of a site-specific transcription factor. This view is supported by crystallographic data showing that contacts with the histone octamer are weaker and fewer for the penultimate ~20 base pairs [33].

4. Histone chaperones join the dance

A further manifestation of the dynamic nature of nucleosomes in vitro, in addition to the 'breathing' of the DNA ends discussed above, is the ability of the histone octamer to translocate or 'slide' along the DNA over significant distances ([9], and references therein). Additionally, the transient removal of one or both H2A–H2B dimers from a nucleosome appears to be involved in many vital cellular processes [34] [35–37]. H2A/H2B destabilization also occurs as a consequence of nucleosome sliding catalyzed by several chromatin remodeling complexes [38], and has been shown to facilitate transcription factor binding [39] [40]. It was recently found by several labs that a specific ATP-dependent chromatin remodeling factor, Swr1, is responsible for the replication-independent incorporation of the histone variant H2A.Z into yeast chromatin (reviewed in [41]). The histone H2A–H2B dimer complexes are delivered by the acidic histone chaperone NAP-1. Members of this protein family are found in most eukaryotes where they perform pleiotropic and ill-defined roles in chromatin assembly [42] and cell-cycle regulation [43]. NAP-1 has the ability to bind both the $(H3–H4)_2$ tetramer and the H2A–H2B dimer [44].

We have recently shown that NAP-1 from yeast reversibly removes and replaces H2A–H2B or histone variant dimers from assembled nucleosomes in vitro, resulting in active histone exchange [45]. This indicates a more active role for NAP-1 in shaping chromatin structure than previously assumed (Fig. 3). The ability of yNAP-1 to bind H2A–H2B dimers and $(H3–H4)_2$ tetramers [44] also suggests a 'scavenger' function of yNAP-1, supported by preliminary data from our laboratory. In this view, NAP-1 would also be acting much like a clean-up crew, plucking apart ill-assembled nucleosomes and removing randomly deposited histone sub-complexes from the DNA.

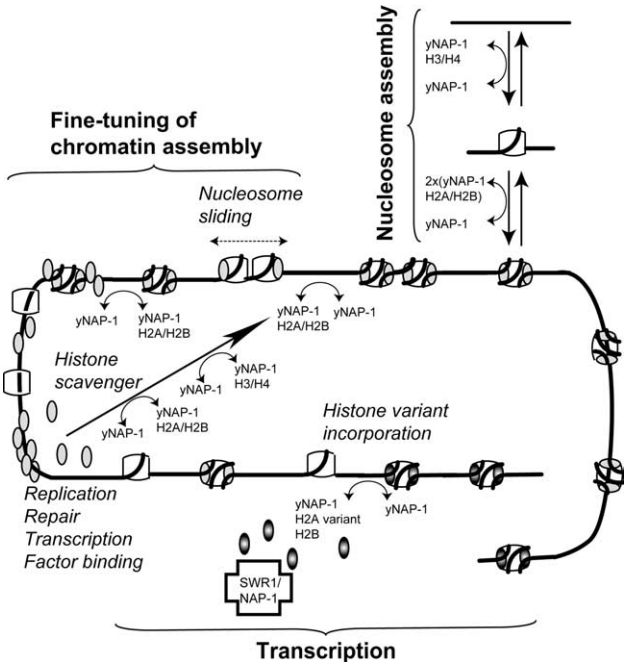

Fig. 3. Schematic showing the different putative roles of the histone chaperone yNAP-1 in chromatin assembly and maintenance. $(H3–H4)_2$ tetramers are shown as white cylinders, H2A–H2B dimers as gray ovals. H2A–variant–H2B dimers are indicated as shaded ovals

Perhaps the most intriguing recently discovered activity for NAP-1 from yeast is that the transient removal of one or both H2A–H2B dimers facilitates nucleosome sliding along the DNA to a thermodynamically favorable position. We showed that NAP-1-dependent histone exchange and nucleosome sliding is independent of ATP and relies on the presence of the C-terminal acidic domain of yeast NAP-1, even though this region (which is the largest of the three acidic regions in this protein) is not required for histone binding and chromatin assembly [45]. This strongly suggests that removal of the H2A–H2B dimer is essential for NAP-1 mediated nucleosome sliding. It remains to be seen whether the temperature-induced sliding observed on many DNA sequences in vitro (see, for example, [17]), and the ATP-dependent nucleosome sliding brought about by the large chromatin remodeling machines also require the transient removal of the H2A–H2B dimer.

5. Conclusions and outlook

Structural studies with nucleosomes containing core histones from different species or non-allelic variants of histone H2A have revealed that the overall nucleosome structure is surprisingly resistant to structural alterations. Sequence differences in strategic regions of the histone fold engender subtle structural

changes at histone–histone interfaces, which in turn may have significant functional implications. The high degree of evolutionary conservation of the major type core histones may therefore have a purpose above and beyond preservation of nucleosome structure.

Chromatin was once viewed as an 'immovable object' that only an advancing replication fork (and possibly an advancing RNA polymerase) can displace [46]. This picture is now being replaced with that of a highly dynamic and malleable assembly that shapes all cellular processes that utilize the DNA substrate, and that is capable of extensive cross-talk with the cellular machinery. Much remains to be learnt on how this is achieved mechanistically, and doubtlessly many activities that are involved in this important aspect of chromatin metabolism remain yet to be discovered. In at least two instances, 'breathing' of DNA ends facilitates the binding of transcription factors to nucleosomal DNA, but further studies are necessary to see whether this is a general phenomenon. Similarly, the role of one particular histone chaperone, NAP-1 (and presumably that of other acidic histone chaperones) is clearly evolving, from being a mere histone escort that manages histone transport into the nucleus before handing its precious cargo over to chromatin assembly and remodeling factors, to a much more glamorous role in maintaining chromatin structure and modulating nucleosome fluidity and dynamics. It remains to be seen whether these properties are also of importance in vivo. A complete understanding of the processes that regulate chromatin structure and dynamics are only possible through a synthesis of structural, mechanistic, and in vivo approaches.

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