New and Notable

DNA Sequence Mediates Nucleosome Structure and Stability

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Nucleosomes form the fundamental repeating unit of eukaryotic chromatin. Subtle modifications in nucleosome structure and histone tails regulate chromatin states; hence, a comprehensive understanding of alterations in nucleosome structure is of fundamental importance in chromatin biophysics. The nature of core histone organization and nucleosome dynamics have been extensively studied using biophysical experiments, multiscale computational models, and simulations ((1,2) and references therein). However, the precise mechanisms of how DNA sequence mediates nucleosome structure and stability remains to be completely understood.

The eviction of H2A/H2B core histones from the histone octamer is known to expose nucleosomal DNA for transcription. In this issue of *Biophysical Journal*, Kelbauskas et al. (3) have investigated the sequence dependence of H2A/H2B eviction mediated by histone chaperone yNAP-1 using single-molecule Förster resonance energy transfer (FRET) and fluorescence correlation spectroscopy (FCS) experiments. Single molecule experiments have gained a newfound interest in probing chromatin dynamics, in part, because of their ability to study subtle variations in chromatin dynamics at a single nucleosome level, rather than probing population-averaged properties (1,4–6). H2A/H2B dimers interact with nearly 30 basepairs of nucleosomal DNA, thus depletion of H2A/H2B dimers from the nucleosomes results in significant exposure of the nucleosomal DNA. Understanding H2A/H2B depletion is of special significance in chromatin biophysics as loss of H2A/H2B histones is associated with important cellular processes, including transcription elongation, ATP-dependent nucleosome remodeling, and function of RNA polymerase.

In a previous study (7), Kelbauskas et al. used FRET and FCS assays to study the sequence dependence of mononucleosome structure and stability of chromatin reconstitution in bulk solution conditions. This sequence dependence was tested in nucleosomes having three physiologically relevant DNA sequences: 1), sea urchin 5S rDNA; 2), DNA from yeast *GAL10* promoter sequence; and 3), DNA Mouse Mammary Tumor Virus promoter DNA (MMTV-B). While *GAL10* and MMTV-B nucleosomes are known to exhibit significant conformational dynamics associated with gene activation in vivo, sea urchin 5S rDNA is extensively used as a template DNA for in vitro studies of nucleosome dynamics. Nucleosome stability was tested by analyzing the conformation-dependent FRET response in the three nucleosomes, with FRET donor and acceptor fluorophores attached to nucleosomal DNA.

The authors showed that upon dilution to subnanomolar concentrations (corresponding to single-molecule studies), the efficiency of FRET signals from MMTV-B and *GAL10* nucleosomes is reduced by as much as 35%, while that from 5S nucleosomes remains undiminished. The in vitro FCS assays showed that the relative diffusion coefficient for 5S nucleosomes was higher than that of MMTV-B and *GAL10* nucleosomes, suggesting that under ambient conditions, 5S nucleosomes are more compact, as compared to MMTV-B and *GAL10* nucleosomes. Similar results are observed for the three sets of nucleosomes at elevated temperatures (42°C) and upon treatment with high salt concentrations. These observations suggest that 5S nucleosomes are more stable than MMTV-B and *GAL10* nucleosomes. In coherence with genomic regulatory mechanisms, these differences in nucleosome structure and stability may mediate nucleosome recognition and histone modifications.

In this issue, Kelbauskas et al. (3) report that treatment with histone chaperone yNAP-1 causing H2A/H2B histone eviction leads to the same FRET changes as observed in corresponding subnanomolar dilution assays. Notably, in both assays, the extent of reduction in FRET signal was also similar for MMTV-B and *GAL10* nucleosomes. Furthermore, salt stability assays suggest that while yNAP-1 histone chaperone was acting on each of the three nucleosomes, 5S nucleosomes exhibit no FRET change. These results reinforce the previous studies that H2A/H2B release causes sequence-dependent FRET responses. The yNAP1 assay also suggests that the three mono-nucleosomes follow two-state dynamics with folded and unfolded states. While the two-state behavior is most pronounced in 5S nucleosomes, the rate of nucleosomal DNA dynamics is enhanced in *GAL10* and MMTV-B nucleosomes. Simulations of a nucleosome core particle having *Xenopus laevis* core histones and a palindromic DNA from human α-satellite sequence (2) also suggest specific interactions between H2A-H2B histones and nucleosomal DNA. Functionally important interactions are reported at interface of H4-H2A and H4-H2B histones (2), suggesting that the sequence-dependent eviction of H2A-H2B dimers may also be evolutionarily conserved in higher eukaryotes.

Kelbauskas et al. (3) analyze two possible hypotheses for the biophysical mechanism leading to differences in FRET response in these nucleosomes under subnanomolar concentrations: the first hypothesis suggesting differences

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in the amount of H2A/H2B released, while the second hypothesis suggests that H2A/H2B-depleted 5S nucleosomes are able to keep the fluorophores closer than MMTV-B and *GAL10* nucleosomes, resulting in high FRET efficiency. The first hypothesis is unlikely based on experiments on loss of nucleosomal FRET efficiency at subnanomolar concentrations. The second hypothesis is supported by yNAP-1 and nucleosome heating assays, where similar extent of H2A/H2B release is observed for the three nucleosomes, while 5S nucleosomes have higher FRET efficiencies. These results suggest that differences in FRET response are mediated by differences in DNA-histone binding abilities in H2A/H2B-depleted nucleosomes.

Previous FCS experiments from the Bustamante group performed on nucleosomes consisting of synthetic highly-positioned 601 DNA sequence (6) are consistent with the results from Kelbauskas et al. (3), performed with nucleosomes having the three natural DNA sequences. Furthermore, recent studies of 601 and related sequences were performed by Gansen et al. (4), who have expanded the sites labeled on nucleosomes for FRET studies as well as investigated the binding of linker histone H1. Using FCS, they were able to distinguish between a 601 sequence that has a couple of nucleosome positions and another sequence that positions more precisely.

It is important to highlight the hypotheses emerging from these studies. The 5S DNA is tightly bound to the H3-H4 tetramer, as compared to the MMTV-B and *GAL10* DNA. While core histones are among the most conserved proteins in the proteome, core histone variants (H2A.Bbd, H2A.Z, H2A.X, macroH2A, H3.3, CENP-A) are expressed genome-wide, throughout the cell-cycle, substituting the wild-type histones and thereby causing structural and chemical heterogeneity in chromatin. This heterogeneity imparted by core histone variants can dynamically alter the nature of direct and water-mediated hydrogen bond and salt-bridge interactions formed between nucleosomal DNA and variant histones, thereby mediating the structure and stability of chromatin at the genomic scale. DNA sequence-dependent nucleosome stability may be synergistic with changes in DNA-protein interactions occurring in nucleosomes having variant histones. For instance, the *Saccharomyces cerevisiae* centromeres are localized at the conserved CEN DNA sequence and contain CENP-A variant histones, Cse4p substituting H3 at the centromeric nucleosome. The Cse4p makes extensive salt-bridge interactions with the CEN-DNA sequence, resulting in a bent cruciform-like topology at the kinetochore junction (8). These sequence-dependent interactions of CEN DNA with Cse4p may be essential for centromere organization and stabilizing the structure of centromeric nucleosomes against microtubular stress. The example also suggests a general DNA sequence-dependent mechanism for regulating chromatin states, whereby differences in biophysical properties of nucleosomes caused by sequence-specific interactions of nucleosomal DNA with wild-type versus variant core histones alter the euchromatic and heterochromatic nature of chromatin.

Recent experiments and computational models (9,10) have also suggested that nucleosome positioning is regulated, in part, by the constituent DNA sequence, resulting in a genomic code for nucleosome positioning (10). The DNA-sequence dependent nucleosome stability observed by Kelbauskas et al. also suggests a model for the genomic code of nucleosome positioning, wherein core-histones bound to relatively destabilized nucleosomal DNA transiently slide to genomic loci having stronger histone-DNA interactions resulting in stable, highly-positioned nucleosomes. Presence of such sequence-dependent nucleosomal sliding to more stabilizing loci genomic may be tested using a FRET assay similar to the one described in the Kelbauskas et al. (3), with FRET probes attached to high-positioning flanking linker DNA. These studies suggest a genomic role in nucleosome stability, mediated by sequence-dependent alterations in histone-DNA interactions.

While the precise mechanism of how DNA sequence-dependent nucleosome stability results in changes in chromatin dynamics remains to be completely understood, a possible role of DNA sequence-dependent nucleosome stability on occupancy of nonallelic histone variants recruited at distinct chromatin loci presents several interesting hypotheses. Overall differences observed in histone-DNA interactions for the three DNA sequences suggest that the kinetics of spontaneous unwrapping of nucleosomal DNA (6) may depend on the underlying nucleotide sequence. Furthermore, these sequence-dependent changes may also mediate the extent and kinetics of chromatin remodeling. Chromatin organization is influenced by the nature of interactions between core-histones and nucleosomal DNA. However, these interactions may, in part, be mediated by changes in conserved variant histones as well as changes in genomic DNA sequence. The substitution of wild-type core histone with variants is known to occur throughout the cell cycle. The recruitment of variant histones may be dependent, in part, on the nucleosomal DNA contexts, thereby regulating chromatin states at genomic levels. Understanding such synergy between DNA-sequence-dependent chromatin stability and chromatin heterogeneity caused by core histone variants may be central to mechanistic understanding chromatin structure at genomic scales. Clearly, genome-wide analyses of nucleosome-positioning and wild-type/variant core histone occupancy should aid in understanding the dynamics of chromatin organization.

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