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# Regulation of nucleosome stability as a mediator of chromatin function

Paul G Giresi, Mayetri Gupta and Jason D Lieb

Interactions among regulatory proteins, enzymes and DNA are required to use the information encoded in genomes. In eukaryotes, the location and timing of interactions between these proteins and DNA is determined in large part by whether DNA at a given locus is packaged into a nucleosome. Given the central role of nucleosome formation in regulating genome function, many mechanisms have evolved to control nucleosome stability at loci across the genome. New conclusions about the role of the DNA sequence itself in the regulation of nucleosome stability have come from two new types of experiment: high-resolution mapping of *in vivo* nucleosome occupancy on a genomic scale; and *in vivo* versus *in vitro* comparisons of nucleosome stability on natural DNA templates. These new types of data raise intriguing questions about the evolutionary constraints on DNA sequence with regard to nucleosome formation, and at long last might enable the derivation of DNA sequence-based rules that produce reliable predictions of nucleosome behavior.

## Addresses

Department of Biology and Carolina Center for Genome Sciences and Department of Biostatistics and Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA

Corresponding author: Lieb, Jason D (jlieb@bio.unc.edu)

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## Introduction

In eukaryotic cells, the activity of all DNA-templated processes, which include transcription, recombination, repair and replication, are controlled in part through the regulation of chromatin composition. This realization has been crucial to understanding genome function, because control of chromatin enables cells to control the use of information stored in DNA. The most basic organizational unit of chromatin is the nucleosome, which is composed of DNA and an octamer of histone proteins. New types of data generated over the past two years

strengthen the argument that one of the most important aspects of chromatin regulation involves modulating the stability of nucleosomes.

One may define ‘nucleosome stability’ broadly to refer to the population of intact nucleosome–DNA complexes at a given locus relative to the population of those in an unfolded or disrupted state. This definition encompasses histone–histone, histone–DNA and nucleosome–nucleosome interactions. In general, chromatin creates an environment that is not permissive to enzymes and accessory proteins that require direct access to DNA, and there is mounting evidence that regulated differences in nucleosome stability have significant biological consequences [1,2]. It is perhaps not surprising, then, that there are many biological mechanisms through which nucleosome stability is regulated (Figure 1). These include regulation of nucleosome deposition following DNA replication [3]; the activity of nucleosome-remodeling complexes such as SWI–SNF and RSC [4]; binding of regulatory proteins to DNA [5]; transcriptional initiation and elongation by RNA polymerase II [6]; incorporation of histone variants; post-translational modification of histones [7]; and inherent properties of DNA sequence [8•]. In living cells, these mechanisms for regulation of nucleosome stability are intertwined in complicated ways. However, it is convenient conceptually to divide them into two categories: (i) ‘intrinsic’ mechanisms mediated by molecules that comprise the nucleosome itself and affect its stability, for example the effects of DNA sequence and some histone modifications and variants; and (ii) ‘extrinsic’ mechanisms that act *upon* nucleosomes to regulate their stability and are mediated, for example, by the transcriptional apparatus, remodeling enzymes, and chaperones (Figure 1). Many of these mechanisms are being reviewed by others in this issue of *Current Opinion in Genetics & Development*, so we discuss only intrinsic mechanisms of regulating nucleosome stability, with a focus on DNA sequence.

## Evidence for DNA sequence as a major determinant of nucleosome-positioning and occupancy *in vivo*

Defined DNA templates have long been used for the *in vitro* reconstitution of nucleosomal chromatin. In many cases, these templates were originally chosen for their ability to create well-ordered, reproducible nucleosome positions [9,10]. For example, the 200 bp ‘Widom 601’ DNA sequence (see Glossary) forms arrays of ordered nucleosomes when placed in tandem head-to-tail arrangements and was originally selected in a screen

**Glossary**

**Hidden Markov model:** A probabilistic model that allows the state of the system at a given point to be influenced by the state at the previous point. The complexity is that the process being modeled cannot itself be observed. Instead, a sequence of noisy measurements generated from the process is used to reconstruct (estimate the parameters of) the process of interest.

**Hydroxyl-radical footprinting:** Hydroxyl (OH) radicals are reactive oxygen species that cleave DNA at the exposed minor groove, yielding a cleavage pattern that follows the helical periodicity of the DNA molecule. The cleavage pattern of nucleosomal DNA allows the preferred nucleosome binding sites and rotational position of DNA fragments to be determined.

**Inference:** The act of drawing conclusions about the properties of an unknown distribution from data generated by that distribution.

**Predictive model:** A statistical model that allows prediction of the behavior of a system.

**Widom 601 DNA sequence:** A sequence identified from a large pool of random synthetic DNA as having a high affinity for the histone octamer, and the ability to position nucleosomes *in vitro*.

for DNA sequences with a high affinity for nucleosomes [11]. The existence of these *in vitro* DNA templates clearly demonstrates that DNA sequences are capable of precisely positioning nucleosomes in the absence of any co-factors.

DNA sequence analysis of *in vitro* DNA templates, along with analysis of sequence from *in vivo* loci for which nucleosome positions have been precisely mapped, revealed that positioning is influenced by dinucleotide and trinucleotide patterns [12]. Distances between major maxima of the AA and TT positional distributions have been shown to display a periodicity of about 10.4 bases in nucleosomal DNA [13]. One conjecture is that the precise positioning of AA and TT dinucleotide endows the DNA duplex with the flexibility required to conform to nucleosome structure [13]. Although much effort has concentrated on determining dinucleotide frequencies and other simple sequence motifs that correlate with nucleosome positioning [13–15], DNA-encoded positioning signals are relatively weak and difficult to detect in any individual nucleosomal sequence. Therefore, the question of whether sequence plays a major role in nucleosome positioning and occupancy across the genome in living cells has been a matter of vigorous debate.

Two different types of experimental data were needed to accelerate the resolution of this debate. First, given that there is not enough signal in the sequence surrounding any one nucleosome position to know which bases are important for that positioning event, data from many *in vivo* positioning events on natural DNA templates were needed so that rules could be derived from a large population of interactions. Second, to directly measure the contribution of DNA sequence, there was a need to precisely map nucleosome positions at a natural locus *in vivo*, and then to test how much of that pattern could be recreated with only DNA and histones *in vitro*. In 2004 and 2005, data of both types were published.

**Chromosome-scale *in vivo* nucleosome-positioning data**

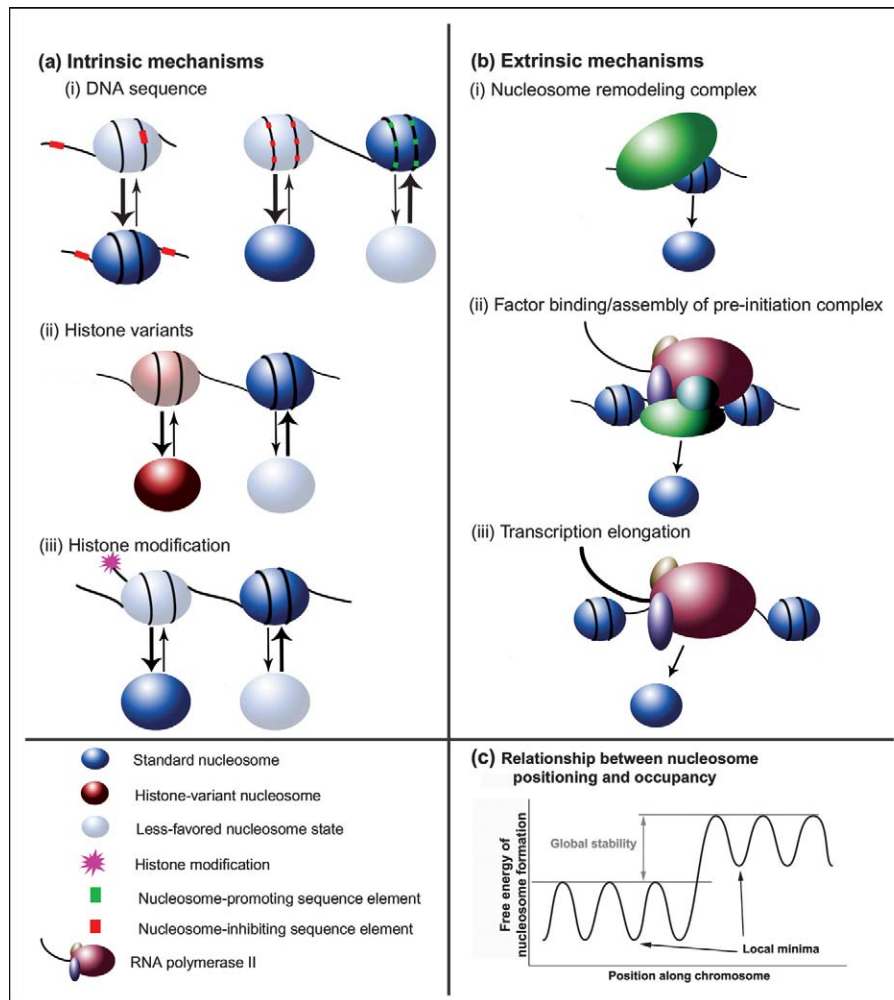
Data defining a large population of nucleosome–DNA interactions have been generated through the use of DNA microarrays combined with chromatin immunoprecipitation (ChIP) and nuclease-protection-based assays. Early lower-resolution microarray-based studies of genome-wide nucleosome occupancy in *S. cerevisiae* (hereafter ‘yeast’) indicated that there was an inherent, transcription-independent difference in nucleosome occupancy between coding and non-coding DNA sequence [8<sup>••</sup>,16,17<sup>•</sup>]. In general, coding sequence was found to be more stably associated with nucleosomes than was non-coding sequence. Subsequent studies using a nuclease-based strategy to map nucleosome positions, either by traditional methods [18] or with a higher-resolution microarray covering a single yeast chromosome [19<sup>••</sup>], agreed with the earlier data [8<sup>••</sup>,16], and additionally showed that ~65% of the nucleosomes were well-positioned. That such a high proportion of nucleosomes were stereotypically positioned came as somewhat of a surprise, considering that the data was derived from a large population of asynchronous cells, and that previous data had indicated that nucleosomes exhibited little thermodynamic preference for one DNA sequence over any other [20].

Analysis of the relationship between DNA sequence and nucleosome positioning revealed that multiple stretches of polyA or polyT sequences were associated with the nucleosome-depleted regions of promoters and had an increased likelihood of occurring in ‘linker’ DNA, which was defined as any sequence that was not nucleosomal. These effects were independent of overall AT composition [19<sup>••</sup>]. This analysis is consistent with the notion that long stretches of polyA or polyT DNA are less amenable to nucleosome formation (Figure 1). The DNA sequence within the nucleosome-free regions of promoters was well-conserved among seven species of yeast. This conservation extended beyond shared transcriptional regulatory motifs and provides evidence that the sequence associated with nucleosome positioning at promoters has functional importance.

**Reproduction of *in vivo* nucleosome occupancy and positioning *in vitro***

To test how closely *in vivo* nucleosome occupancy and positioning could be recreated with only DNA and histones *in vitro*, the *HIS3*, *PET56* and *DED1* loci were investigated in detail using a novel ‘nucleosome-scanning’ assay [8<sup>••</sup>]. Nucleosomes reconstituted on natural promoter sequences *in vitro* exhibited a pattern of occupancy and positioning that closely mirrored the *in vivo* situation. The bi-directional *HIS3–PET56* promoter is nearly devoid of nucleosomes *in vivo*, and the inability of the promoter DNA sequence to be packaged into nucleosomes was nearly perfectly reproduced *in vitro*.

Figure 1



Mechanisms for regulating nucleosome stability. Processes involved in modulating nucleosome stability can be divided into intrinsic and extrinsic mechanisms. **(a)** Intrinsic mechanisms affect the stability of the nucleosome itself. These mechanisms can be thought of as affecting the thermodynamic binding preference of a nucleosome for a given genomic locus. Nucleosomes can adopt a favored, stable state (darker nucleosomes) or an unfavored, unstable state (lighter nucleosomes). (i) Inhibitory sequence elements, such as polyA (red boxes), have been observed at nucleosome boundaries, presumably influencing positioning, and in extended nucleosome-depleted regions. There is evidence that periodic positioning of certain nucleotides (green dots), provides the flexibility required to produce a stable nucleosome, whereas others are inhibitory (red dots). (ii) Incorporation of histone variants (red nucleosome) alters the interactions between histones, possibly stabilizing or destabilizing nucleosomes for specific tasks. (iii) Some post-translational modifications, such as histone acetylation, reduce the energetic cost of removing a nucleosome. **(b)** Extrinsic mechanisms involve proteins that act upon nucleosomes to alter their stability. These mechanisms can be thought of as affecting the *kinetic* stability of chromatin, rather than affecting the thermodynamic stability. (i) Nucleosome-remodeling complexes can move or displace nucleosomes. (ii) Nucleosomes 'breathe', periodically entering a partially unwrapped state. Binding of regulatory proteins to DNA can block the formation of nucleosomes by stabilizing a disrupted state, and once bound can inhibit nucleosome formation through exclusion [36\*,37,38]. Assembly or initiation of the transcriptional machinery can also facilitate nucleosome clearance at promoters. (iii) Transcriptional elongation promotes the removal of nucleosomes. **(c)** Nucleosome occupancy versus nucleosome-positioning. A schematic representation of hypothesized nucleosome-positioning by local energy minima. The concept of occupancy or stability can be thought of as positioning at a larger scale. Occupancy and positioning are therefore somewhat independent and can be determined by layered interactions between both intrinsic and extrinsic mechanisms that alter the energy required for nucleosome formation at different scales.

The authors also demonstrated that paucity of nucleosomes at the *HIS3-PET56* promoter, and the positioning of nucleosomes within the coding regions of *PET56* were maintained when the DNA was introduced to the evolutionally distant species *Schizosaccharomyces pombe*.

The study by Sekinger *et al.* [8\*\*] also suggests that nucleosome occupancy is dictated by sequence at a very local level. Progressive deletion of promoter DNA did not affect positioning of downstream nucleosomes in the coding regions but did slowly increase nucleosome

occupancy at the promoter. Increases in nucleosome occupancy were shown to be directly correlated with decreases in accessibility of the underlying DNA template. These data suggest a cumulative rather than discrete effect of DNA sequence [8\*\*] and extend evidence from previous studies [21] that suggested that preferential accessibility of promoters is determined by a local property of DNA sequence. The intrinsic preferences of histones for DNA sequences might be responsible for establishing the baseline chromatin landscape, which is then fine-tuned by extrinsic factors such as nucleosome-remodeling complexes.

### Developing reliable computational predictions of nucleosome occupancy and positioning

The influence of DNA sequence on nucleosome occupancy appears to occur on the scale of a single nucleosome (~200 bp) and to be cumulative and diffuse. Therefore, both a detailed biophysical understanding of nucleosome–DNA interactions and sophisticated statistical tools are likely to be required to predict nucleosome occupancy and position from DNA sequence.

### Biophysical predictions of nucleosome-positioning sequences

The growing body of nucleosome-position data is challenging conventions, particularly previous models concerning the flexibility of DNA. The persistence length of a given DNA sequence, which can be thought of as the length of the polymer for which there is a negligible energetic cost of bending, is thought to be inversely correlated with the likelihood of that DNA sequence participating in nucleosome formation [22]. Recent experiments have shown that short DNA fragments are more flexible and have lower persistence lengths than biophysical models would predict, and that sequences found in well-positioned nucleosomes were more flexible than random sequences of comparable length [23\*].

The relationship among flexibility, occupancy and positioning was investigated further by altering the flexibility of natural DNA, and by testing its nucleosome-formation potential [22]. This was accomplished either by substituting 2,6-diaminopurine (DAP) for adenine, which made DNA less flexible, or by substituting inosine for guanosine, which made DNA more flexible. Introduction of DAP into DNA forces a purine two-amino group into the minor groove and, as expected, increased the persistence length of fragments from 40% to 70%. In all of the cases in which DAP was introduced to DNA, affinity for nucleosome formation was decreased. It must be noted, however, that the presence of a B-form DNA structure after DAP substitution was not confirmed. In experiments that aimed to increase DNA flexibility through inosine substitution, the persistence length of DNA was reduced as expected, and in all but one case the altered DNA showed

increased affinity for nucleosomes. The lone exception was postulated to have resulted because the inosine substitution altered the inherent curvature of the DNA, as measured by hydroxyl-radical footprinting (see Glossary). Remarkably, the same DNA sequence whose curvature was altered upon inosine substitution was also shown to be required for the formation of a second adjacent nucleosome *in vitro* [24]. This observation indicates that the formation of a stable nucleosome at a given position might be dependent on the structural aspects of the DNA in a neighboring nucleosome.

### Statistical predictions of nucleosome-positioning sequences

Several sequence elements that are involved in nucleosome positioning have been identified using computational approaches. Regular periodic oscillations of sequence signals composed of combinations of A and T, and a 'VWG' triplet [(non-T)(A/T)(G)] element, occur specifically in regions of murine DNA that formed ordered nucleosomes *in vitro* [13,25]. Nucleosome formation tended to be inhibited in DNA regions that had low counts of the oscillating VWG signal (with a period ≈10 bp), presumably because they were less flexible [26]. These and related results [13,19\*\*] suggest that it may be possible to computationally predict genomic regions that have distinctive chromatin structures.

RECON is one software package that attempts to predict nucleosome position using statistical tools, nucleosome position data, and prior knowledge about sequence elements that are likely to be important for positioning. RECON predicts a nucleosome-formation potential profile over any given sequence, on the basis of a model derived from the dinucleotide counts in a training set of sequences from the Nucleosomal DNA Database ([ftp://ftp.ebi.ac.uk/pub/databases/nucleosomal\\_dna/](ftp://ftp.ebi.ac.uk/pub/databases/nucleosomal_dna/)) [27]. As with RECON, most of the current approaches to understanding how nucleosome-positioning is influenced by DNA sequence have been based on *inference* (see Glossary), using rules based on pre-selected sequence factors [13,25]. However, the ultimate goal is to build a *predictive* model (see Glossary) that can establish how nucleosomes will form depending solely on DNA sequence.

If the underlying DNA sequence indeed has a strong deterministic effect on nucleosome positioning, the genome might harbor a nucleosome-positioning code. Similar to related challenges such as discovering transcription-factor binding sites, the problem of deciphering the nucleosome-positioning code is likely to require many iterations of close collaboration between experimental and computational researchers. The most difficult step will probably be understanding and modeling how the discovered sequence signals are coordinated to influence nucleosome position. The difficulty arises because nucleosome positions are necessarily correlated — the

placement of one nucleosome imposes restrictions on others in the neighborhood. Hidden Markov models (see Glossary) are powerful and flexible probabilistic tools for capturing local dependencies and matching patterns, and they are likely to play a lead role in building verifiable models of nucleosome positioning [19<sup>••</sup>,28]. With advances in genome-wide nucleosome detection, the task of comparing sequence-based predictions with experimental results is already feasible [17<sup>•</sup>,19<sup>••</sup>].

## Conclusions

There is not sufficient information contained in linear DNA sequence — as it is conventionally conceived — to specify cellular or developmental function. ‘Sequence-specific’ DNA-binding proteins recognize short, degenerate motifs that occur thousands of times throughout the genome. Additional information, beyond that provided by DNA sequence, is required to guide DNA-binding proteins to appropriate loci [8<sup>••</sup>,19<sup>••</sup>,29,30]. The regulation of nucleosome occupancy through intrinsic and extrinsic mechanisms is likely to provide much of this extra information by regulating access to the DNA template [1,31–34]. The ‘outsourcing’ of information from the level of short-sequence elements on linear DNA to accessory packaging proteins is a hallmark of organismal complexity, and may be a consequence of the difficulty in specifying the multitude of biochemical interactions required for genome function with just four nucleotide bases.

New types of data suggest that DNA-sequence properties are a major determinant of intrinsic nucleosome-stability. The putative role of DNA sequence in nucleosome organization, which appears to occur on a scale of ~200 nucleotides rather than on the codon-based 3-nucleotide scale recognized for coding sequence, will require new ideas about what it means for a sequence to be ‘conserved’. A similar conceptual shift was recently formalized for the conservation of short DNA-binding motifs [35]. A directly related challenge involves recognition of the constraints that nucleosomal packaging imposes on DNA sequence evolution. Cumulatively, across a genome, these constraints might influence DNA sequence evolution to a degree that exceeds the constraints imposed by coding sequence. For these constraints to be measured, accurate models of how any given change in DNA sequence will affect nucleosome formation must be generated. Data similar to that highlighted here should enable the identification of sequence properties positively and negatively associated with nucleosome formation, and this might lead to accurate models of nucleosome formation for any given DNA sequence.

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Using a high-resolution microarray covering most of the yeast genome, the authors determined general nucleosome-occupancy and the pattern of post-translational modifications of histones, particularly methylation and acetylation. Lower nucleosome-occupancy is observed in the majority of intergenic regions relative to that in coding regions, and highly transcribed regions are nucleosome-depleted relative to regions of lower transcriptional rates. Both observations are in agreement with previous low-resolution data. These findings emphasize the importance of the regulation of nucleosome stability in transcriptional regulation.

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