Nucleosome positioning

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Abstract

Genomic nucleosome positions determine DNA accessibility to regulatory molecules, and thus modulate gene expression. Nucleosome positioning can be characterised by the location of the nucleosome centres, average occupancy landscapes, the stability of individual nucleosomes, and integral parameters such as the nucleosome repeat length. Nucleosome positioning depends on the DNA sequence affinity of the histone octamer, statistical positioning of nucleosomes by genomic boundaries, chemical modifications of DNA or histones, abundance of linker histones, competitive binding of transcription factors, and nucleosome repositioning by chromatin remodellers. The quantitative description of nucleosome positioning depending on the cell type/state has been addressed using a number of approaches, and this problem still poses significant challenges.

Keywords

Chromatin, chromatin remodellers, DNA-protein binding, histone, lattice model, nucleosome, nucleosome positioning, statistical positioning, sequence-specific binding

1. Introduction

The genome of a eukaryotic cell is stored inside the nucleus in the form of the nucleoprotein complex called chromatin. If one would gently remove chromatin from the human cell nucleus, the ~2 meter long DNA would appear as a string with beads (nucleosomes). Each nucleosome consists of two copies of each of histones H2A, H2B, H3 and H4 (so called histone octamer) and about 147 DNA base pairs (bp) wrapped around these histones (Luger et al, 1997). The distances between neighbouring nucleosomes can vary from zero to tens of base pairs (van Holde, 1989). Nucleosomes can be formed at any location along the DNA, but their positioning in the genome is not random – it is affected by the DNA sequence and other factors described below, which allow the same genome to be packed differently depending on the cell state. Nucleosome positioning determines the accessibility of DNA to transcription factors and other proteins, and is thus an important regulator of gene expression. Therefore, nucleosome positioning has been an active area of research since the discovery of the nucleosome (Kornberg, 1974; Olins & Olins, 1974). These investigations have further intensified in the 2000s with the developments of new methods allowing direct measurements of genome-wide nucleosome locations using high-throughput sequencing (Ioshikhes et al, 2006; Segal et al, 2006; Yuan et al, 2005). Nowadays nucleosome positioning studies have advanced up to the level of human patients, down to single cells and cell-free DNA in blood (Snyder et al, 2016).

2. Parameters characterizing nucleosome positioning

The regulation of DNA accessibility by nucleosome positioning is performed through different mechanisms that can be characterised by the following four major parameters:

2.1 Nucleosome dyad positions. This term refers to the position of the centre of the DNA segment symmetrically wrapped around the histone octamer (so called dyad), that can be directly measured *in vitro* or inferred from averaging in high-throughput sequencing experiments. Thus, the term "nucleosome positioning" can be understood either in its general sense (including all the parameters listed below), or in a narrow sense, referring to the location of the nucleosome dyads (see Figure 1A).

2.2 *Nucleosome occupancy*. This term refers to the probability of a given DNA site to be occupied by a histone octamer. It can be inferred from averaging over an ensemble of cells studied via Next Generation Sequencing (NGS) experiments. In such experiments the nucleosome occupancy at a given locus is reflected by the density of sequencing reads corresponding to the nucleosomal DNA, which determines the shape of the continuous nucleosome occupancy landscape (Figure 1B).

2.3 Nucleosome stability, accessibility and fuzziness. In the context of genomic nucleosome positioning, nucleosome stability is usually determined by comparing the nucleosome occupancy landscape of the same genomic region obtained at different levels of chromatin digestion. The better the correlations between nucleosome landscapes in different experiments the higher the nucleosome stability. Physically, the nucleosome stability is characterised by the energetic cost to partially unwrap the nucleosome (which depends on the DNA sequence, covalent modifications of DNA and histones, and the length of the unwrapped DNA part). This thermodynamic value can be measured directly in single-molecule experiments (Poirier et al, 2008) or inferred indirectly from genome-wide experiments in the living cells. In the latter case the nucleosome stability is usually defined as a measure of its sensitivity to different levels of chromatin digestion (Chereji et al, 2017; Mueller et al, 2017; Teif et al, 2014). One can similarly define the opposite parameter called accessibility, which refers to the probability to make the nucleosomal DNA accessible for protein binding (Mueller et al, 2017). Another related parameter is called nucleosome fuzziness, which is proportional to the standard error of determining the nucleosome occupancy at a given genomic location based on the averaging of several replicate experiments (Vainshtein et al, 2017) (Figure 1C).

2.4 Nucleosome repeat length (NRL). NRL is an integrative parameter equal to the average distance between the centres of neighbouring nucleosomes (Figure 1D). NRL can be calculated either for large enough genomic regions or the whole genome. Genome wide average NRL depends on the cell type and state. For example, it can vary from as small as 160 bp in Yeast to up to 210 bp in human or mouse (van Holde, 1989). NRL also can change during cells differentiation (Teif et al, 2012; van Holde, 1989) and can be different within the same cells in specific genomic regions, e.g. a 10-bp NRL decrease with resepect to genome-average NRL has been reported near bound transcription factors CTCF or SP1 (Teif et al, 2017), and NRLs associated with transcriptionally active and inactive genomic regions are

also known to be different (Valouev et al, 2011).

3. Genetic and epigenetic factors affecting nucleosome positioning

Genomic nucleosome positioning is non-random, and represents a unique characteristic of a given cell state and type. Several counteracting processes affect nucleosome positioning both at the level of the genome (DNA sequence) and epigenome (beyond the DNA sequence). One can distinguish six major determinants of genomic nucleosome positioning:

- Intrinsic DNA sequence affinity of the histone octamer
- Statistical positioning of nucleosomes by genomic boundaries
- Chemical modifications of DNA or histones
- Interaction of nucleosomes with linker histones
- Binding of transcription factors and other chromatin proteins
- ATP-dependent nucleosome repositioning by chromatin remodellers

These factors will be considered in detail in the next sections.

4. Intrinsic DNA sequence affinity of the histone octamer

DNA has a natural affinity for histones as its backbone is constituted by negatively charged phosphate residues and can be neutralised by histones which bear positive charges. Thus, nucleosomes can form at any location, but some locations are more likely to be selected for nucleosome formation for a number of reasons. The geometry of the DNA double helix is characterised by six parameters: twist, shift, slide, roll, rise and tilt (van Holde, 1989). In the first approximation most of these parameters can be determined from the dinucleotide content neglecting the effect of longer than nearest-neighbour nucleotides either based on available crystal structures (Olson et al, 1998) or molecular dynamics simulations (Lankas et al, 2003; Lavery et al, 2010). Therefore, studies of dinucleotide compositions of genomic DNA have contributed significantly to the establishment of the nucleosome positioning field. Almost 40 years ago it was noticed that genomic DNA is characterised by ~10 bp periodicity of dinucleotide distribution, which theoretically could play a role in histone recruitment to specific sites in the DNA (Trifonov & Sussman, 1980). Later such periodicities were indeed observed in the nucleosome core DNA sequences (Satchwell et al, 1986).

In order to find the strongest nucleosome positioning DNA sequence, *in vitro* nucleosome reconstitution via salt-dialysis was performed for a large pool of randomly generated DNA sequences (Lowary & Widom, 1998). As a result of this study the sequence named “601” (later referred to as the “Widom 601” was found to have the highest affinity for the histone octamer. Furthermore, it has been demonstrated by genome-wide sequencing that G/C rich regions have higher nucleosome density, while A/T rich regions are more nucleosome depleted (Segal & Widom, 2009). At the intermediate scale, one can distinguish longer, nucleosome-size motifs, which are either attractive for the histone octamer, such as e.g. the Widom 601 sequence (Lowary & Widom, 1998) and so called Trifonov’s “strong nucleosomes” (Trifonov & Nibhani, 2015), or present nucleosome excluding barriers (Drillon et al, 2016). The cumulative evidence from many experiments conducted in the pre-NGS era confirmed the hypothesis that the DNA sequence at least partially affects the positions of nucleosomes. This hypothesis was further supported when first genome-wide locations of

nucleosomes have been mapped (Ioshikhes et al, 2006; Lee et al, 2007; Segal et al, 2006; Yuan et al, 2005). Later genome-wide studies have revealed that nucleosome positions change with cell differentiation and during the cell cycle, which has led to the refinement of the concept of DNA-sequence dependent nucleosome organization (Schones et al, 2008; Whitehouse et al, 2007). It is currently believed that ~9% of nucleosomes are arranged consistently by the DNA sequence across different types of human cells (Gaffney et al, 2012).

Since the core histone proteins are extremely conserved among different species, the physics that determines DNA sequence preferences for the histone octamer is universal from yeast to human. However, the ensemble of nucleosomal DNA sequences can differ between species, and so are the uses of nucleosomes in gene regulation. For example, nucleosome positioning at functional genomic elements can be either favoured by default (the genomic element is always closed by the nucleosome unless active nucleosome displacement happens leading to the activation of this element), or it may be unfavourable (nucleosome depletion by default allowing TF binding to a given site unless a nucleosome is actively repositioned there). A recent study showed that the former mechanism is probably preferred in higher eukaryotes while the latter is implemented in unicellular organisms (Tompitak et al, 2017). This study reported that promoters of multicellular organisms are in general characterised by nucleosome-favouring sequences, while unicellular organisms have nucleosome-disfavouring promoters.

5. Statistical positioning of nucleosomes by genomic boundaries

In 1988 Kornberg and Stryer proposed that nucleosome positioning, at least to some extent, is governed by the boundary effect: nucleosomes will form ordered arrays next to any physical boundary in the genome (Kornberg & Stryer, 1988). In the post-NGS era genome-wide maps of nucleosome binding have proved this hypothesis. The average nucleosome occupancy shows regular oscillations near genomic locations that act as a boundary, such as the nucleosome depleted transcription start sites (Mavrich et al, 2008), replication origins (Eaton et al, 2010) or large DNA-bound transcription factors such as CTCF (Cuddapah et al, 2009; Fu et al, 2008). Even large genomic intervals such as genes experience boundary effects by their nucleosome-depleted starts and ends (Chevereau et al, 2009). Nucleosomes are different from the analogy of "beads on the string" in that nucleosomes are not freely moving along the DNA. Indeed, nucleosome reconstitution experiments show that the nucleosome occupancy oscillations around TSS can be recapitulated *in vitro* only in the presence of ATP and energy-dependent chromatin remodellers which facilitate nucleosome movements (Zhang et al, 2011). Nevertheless, even with the non-equilibrium component introduced by chromatin remodellers, nucleosome density oscillation near genomic barriers can be quantitatively predicted from equilibrium thermodynamics consistently with the experiments (Beshnova et al, 2014; Mobius & Gerland, 2010; Riposo & Mozziconacci, 2012; Rube & Song, 2014). Interestingly, recent studies show that up to 37.5% of human nucleosome positions can be accounted for by considering boundary effects created by nucleosome-excluding sequences (Drillon et al, 2016).

6. Chemical modifications of DNA or histones

Covalent modifications of DNA and histones represent a large layer of epigenetic regulation (Portela & Esteller, 2010). Most common DNA modifications are cytosine methylation and

hydroxymethylation in the context of CpG dinucleotides ("CpG" means cytosine followed by phosphate followed by guanine). Histone modifications are numerous and may have combinatorial nature (several different amino acids of the same histone can be covalently modified, and different histones in the same nucleosome can carry different modifications). Epigenetic modifications are established by the "writer" enzymes, and recognized by specific "reader" proteins, which modulate the genetic program based on these modifications (Strahl & Allis, 2000). The molecular action of covalent modifications of DNA and histones is often through nucleosome repositioning. A number of statistically defined relations between nucleosome positioning and covalent chromatin modification have been reported, but there seems to be no simple code connecting DNA/histone modifications with the presence/absence of a nucleosome at a given location. For example, the action of DNA methylation is context-dependent – it is rarely seen in CpG islands and when this is observed, it is associated with recruited nucleosomes. Outside of CpG islands DNA methylation is most likely between nucleosomes (Teif et al, 2014). On the other hand, DNA hydroxymethylation can decrease the nucleosome stability. A methylation/hydroxymethylation switch model has been proposed to explain some of the changes in nucleosome occupancy and stability during embryonic stem cell differentiation when an almost 10% fraction of genomic CpGs change their state from hydroxymethylation to methylation, increasing the nucleosome density of the corresponding chromatin regions (Teif et al, 2014). Histone modifications also have characteristic relations with nucleosome positioning. For example, regions with "active" histone modification marks such as acetylation of histone H3 lysine 4 or 9 are characterised by lower nucleosome density, while "inactive" chromatin marks such as methylation of histone H3 lysine 9 or 27 are characterised by higher nucleosome density (Teif et al, 2014).

7. Interaction of nucleosomes with linker histones

Proteins involved in nucleosome positioning are not limited to the core histones composing the nucleosome. Nucleosome packing also critically depends on the linker histones, which belong to a class of basic nuclear proteins binding the DNA entry/exit from the nucleosome and providing a separation and interaction medium between nucleosomes in chromatin (Bednar et al, 2017). It is well established that the abundance of linker histones affects the NRL (Woodcock et al, 2006), which may in turn determine different types of chromatin packing (Bascom et al, 2017; Routh et al, 2008). Usually NRL increases with the increase of linker histone concentration in the nucleus, but it can be also affected by the competition of linker histones with other abundant chromatin proteins (Beshnova et al, 2014).

7. Binding of transcription factors and other chromatin proteins

Transcription factors (TFs), which bind DNA sequence-specifically, can affect nucleosome positioning in a number of ways considered below (see Figure 1, E-J).

7.1 TF competition with histone octamer. The energy of complete nucleosome unwrapping or partial dissociation of the histone core from the DNA is much larger than typical energies of TF-DNA binding. Therefore, for most TFs nucleosomes can be viewed as almost immobile obstacles that do not really compete with TFs on a timescale of usual TF binding to the DNA. If TF/nucleosome arrangement would have been determined by equilibrium thermodynamics,

nucleosome positioning would be determining TF binding and not the other way around (Teif & Rippe, 2010). Only few chromatin proteins such as CTCF have DNA binding energy comparable to that of the histone octamer, allowing them to act as a boundary affecting up to several nucleosomes in its vicinity (Cuddapah et al, 2009; Fu et al, 2008). It is important to keep in mind that the chromatin is never in a true thermodynamic equilibrium, and thus active energy-dependent processes, as well as the kinetics of binding determine the order of events in terms of the nucleosome/TF competition.

7.2 TF binding to DNA partially unwrapped from the histone octamer. Since the nucleosome is not a single entity, it can "breath" by partially uncoiling the nucleosomal DNA. Transcription factors then can bind the nucleosomal DNA, depending on how far their binding site is hidden inside the nucleosome (North et al, 2012). An effect called "collaborative competition" allows two TFs help each other to bind the nucleosomal DNA, because it is easier to the second TF to bind if the first TF is already bound and the DNA is already partially unwrapped from the nucleosome (Polach & Widom, 1996). This can lead to nonlinear effects of nucleosome positioning at regulatory sites such as enhancers (Mirny, 2010; Teif & Rippe, 2011).

7.3 TF-mediated recruitment of ATP-dependent enzymes. Sequence-specific binding of transcription factors can be used as a strategy to recruit to a given site an enzyme that chemically modifies DNA or histones, or actively translocates the nucleosome (Agalioti et al, 2000). Mode details on this effect can be found in the next section.

8. ATP-dependent nucleosome repositioning by chromatin remodellers

Random sliding of nucleosomes along the DNA is very slow at physiological conditions, and this effect is mostly limited to *in vitro* studies (Meersseman et al, 1992), while nucleosome repositioning in live systems is usually an active process requiring ATP-dependent molecular motors, so called chromatin remodellers (Clapier et al, 2017). For example, it has been shown that the oscillatory nucleosome density pattern near Yeast promoters can be recovered by *in vitro* nucleosome reconstitution on the same DNA sequences only in the presence of the cell extract and added ATP (Zhang et al, 2011). In the absence of ATP the remodeller activity is abolished and nucleosome positioning tends to reflect the thermodynamically favoured pattern. Remodellers can act both in favour and against the thermodynamically preferred pattern depending on the context. A simplistic mathematical representation of remodeller rules is that a remodeller randomly binds a nucleosome, and then depending on the remodeller type, DNA sequence and chromatin context it either moves the nucleosome left/right, or evicts it completely (Teif & Rippe, 2009). Remodellers usually move nucleosomes in discrete steps such as 5 or 10 base pairs along the DNA, which is explained by the mechanics of the double helix repositioning along the nucleosome through formation of small loops relocated along the histone octamer (Clapier et al, 2017). In many instances remodellers bind their target nucleosome non-randomly, and their recruitment is achieved by multiple remodeller subunits that recognize certain TFs or histone modifications (Ho & Crabtree, 2010).

9. Theoretical approaches to predict nucleosome positioning

Computational algorithms for predicting nucleosome positions can be roughly categorized into biophysical (taking into account physical properties of DNA and histones), bioinformatical (learning the rules of preferred nucleosome distributions without knowing details of molecular interactions), and hybrid models representing the mixture of these two approaches. A list of more than 20 web servers that offer nucleosome positioning prediction has been compiled elsewhere (Teif, 2016).

In typical bioinformatics-inspired models no assumptions are made about the underlying forces that determine nucleosome positions. Instead, features of the DNA content are analysed for available experimental nucleosome positioning datasets and it is assumed that nucleosome positioning in other systems follows the same rules. For example, the nucleosomal and linker DNA sequences can be collected from experiments in order to be able to find some common themes in these genomic features, and then analysed using e.g. wavelet analysis to detect changes in di-nucleotide frequency allowing to distinguish between linker and nucleosomal sequences (Yuan & Liu, 2008). The problem with training a model based on nucleotide content is that there are 4^{147} possible arrangements within a 147 bp DNA window, which is larger than the length of any known eukaryotic genome. Even if the model is trained on many datasets from different genomes, these still do not cover all possible nucleotide combinations that can be theoretically encountered. For this reason, models trained on one species are in general expected to underperform on other species (Kaplan et al, 2009), although few universal nucleosome sequence combinations are also expected (Trifonov & Nibhani, 2015).

In typical biophysical models the prediction of nucleosome positioning is based on available nucleosome crystal structures to infer bending energies corresponding to all dinucleotides and then calculate the nucleosome score for each sliding window of 147 bp with a given dinucleotide distribution. In contrast to bioinformatics models which are trained on data that may or may not be representative of all genomic sequences, biophysical models are based on the assumptions about the underlying molecular dynamics of the process of nucleosome formation (Chereji & Morozov, 2015; Chevereau et al, 2009; Stolz & Bishop, 2010; Tompitak et al, 2017). Most biophysical models are quite demanding in terms of the computational power if they perform realistic simulations. The power of these models is that they allow connecting intrinsic DNA sequence affinities with active repositioning scenarios such as TF/nucleosome competition and remodeller action (Teif & Rippe, 2009). Furthermore, biophysical models allow treating such basic phenomena as statistical nucleosome positioning by the boundary (Chevereau et al, 2009), the exclusion of nucleosomes by their neighbours (Segal et al, 2006) and partial nucleosome unwrapping (Teif & Rippe, 2011). In principle, biophysical models allow treating any level of complexity in nucleosome positioning, provided there is a way to parameterise the models based on some experimental data. The latter is frequently becoming the bottleneck.

10. Future Directions

The number of computational algorithms and experimental datasets devoted to nucleosome positioning continues to increase, but the fundamental questions of how to predict nucleosome cell type/state-specific nucleosome re-positioning and the corresponding changes

in gene expression are still not solved. One major complication is that the rules of chromatin remodelling are difficult to derive from experiments. Another complication is that even when the remodeller rules will be determined experimentally, it will be still a challenge to integrate them in the computational models where both equilibrium and non-equilibrium processes coexist. A third complication is the 3D genome structure – nucleosome positioning happening in the 1D is influenced by and influences the 3D chromatin packing. In order to address these challenges one can expect three major directions of the nucleosome positioning field. Firstly, new high-throughput sequencing methods will continue to emerge, with a special emphasis on single-molecule and single-patient studies. Secondly, the gap in theoretical descriptions will require developing new approaches integrating the dynamics of nucleosome positioning in the description of *in vivo* processes. Thirdly, new biophysical models will have to be developed to account for the interplay of the 1D and 3D nucleosome arrangements based on the experimental data on 3D chromatin packing. The latter is already a very active area of research supported by large funding initiatives (Dekker et al, 2017).

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Relevant Websites

http://generegulation.info/index.php/nucleosome-positioning - catalog of nucleosome positioning resources.

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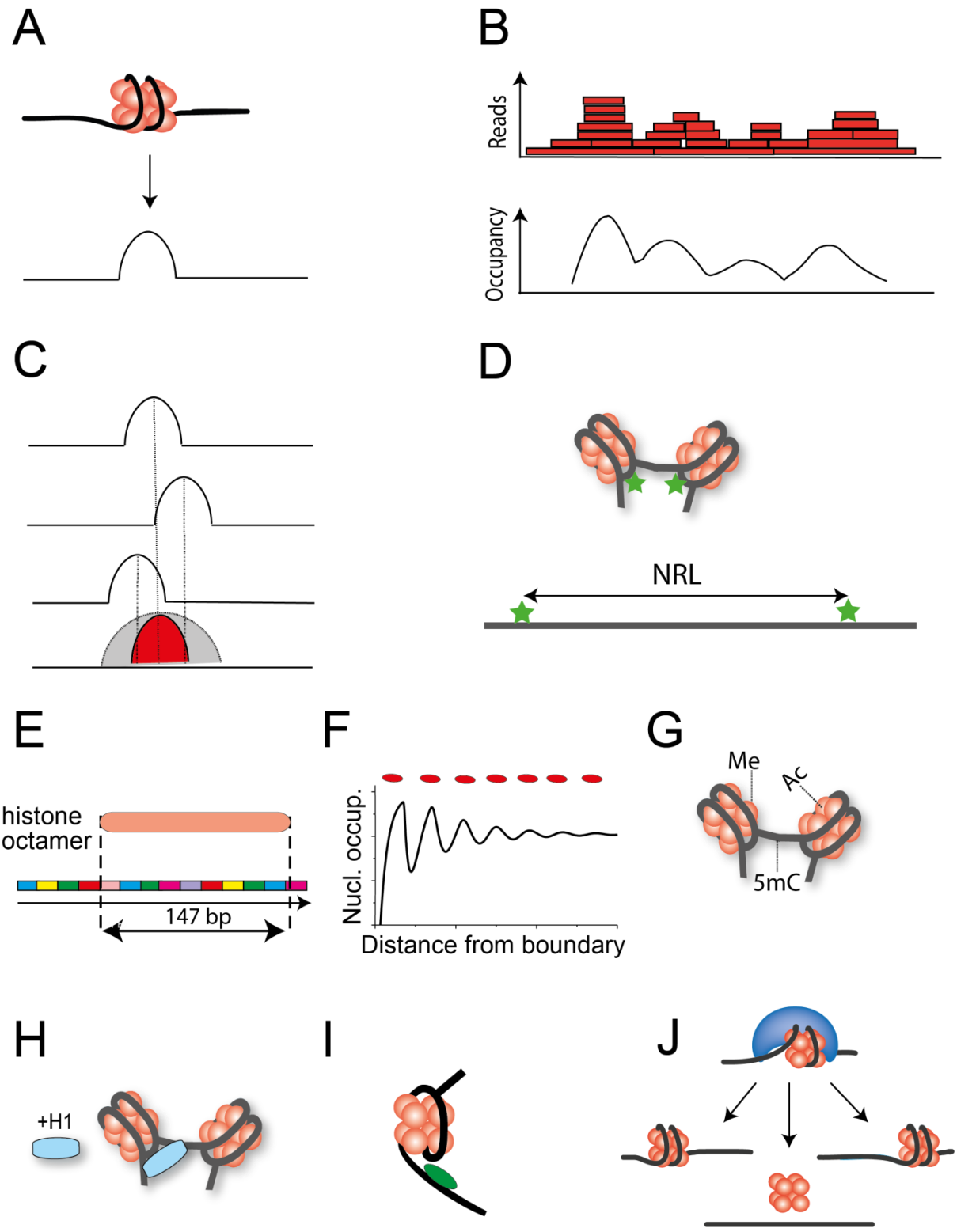
Figures and Tables

Figure 1. Schematic representations of the main characteristics of nucleosome positioning and main factors affecting it. A-D: Main characteristics of nucleosome positioning include the dyad position (A), nucleosome occupancy landscape (B), nucleosome stability/fuzziness (C), nucleosome repeat length (NRL) (D). E-J: Main factors affecting nucleosome positioning include the DNA sequence affinity of the histone octamer (E), statistical positioning by the boundaries (F), covalent modifications of DNA and histones (G), interaction with linker histones (H), competition with transcription factors (I), and action of chromatin remodellers (J).


A
B
Reads
Occupancy
C
D
NRL
E
histone
octamer
147 bp
F
Nucl. occup.
Distance from boundary
G
Me
Ac
5mC
H
+H1
I
J