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Managing the Steady State Chromatin Landscape by Nucleosome Dynamics

Kami Ahmad,[1] Steven Henikoff,[1,2] and Srinivas Ramachandran[3]

[1]Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; email: kahmad@fredhutch.org

[2]Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

[3]Department of Biochemistry and Molecular Genetics and RNA Bioscience Initiative, University of Colorado School of Medicine, Aurora, Colorado, USA

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Abstract

Gene regulation arises out of dynamic competition between nucleosomes, transcription factors, and other chromatin proteins for the opportunity to bind genomic DNA. The timescales of nucleosome assembly and binding of factors to DNA determine the outcomes of this competition at any given locus. Here, we review how these properties of chromatin proteins and the interplay between the dynamics of different factors are critical for gene regulation. We discuss how molecular structures of large chromatin-associated complexes, kinetic measurements, and high resolution mapping of protein–DNA complexes in vivo set the boundary conditions for chromatin dynamics, leading to models of how the steady state behaviors of regulatory elements arise.

Contents

1. INTRODUCTION

The basic unit of chromatin is the nucleosome, where DNA is wrapped around an octamer of conserved histone proteins. Strings of nucleosome particles and linker histones compact the genome in the nucleus. These proteins must be modulated to allow access by DNA-binding proteins at functional locations in the genome. Atomic-resolution structures for the key complexes that compose chromatin and those that navigate DNA make it apparent that these two kinds of complexes will clash (**Figure 1**). Additionally, since progressing transcriptional and replicative polymerases melt DNA in front and reanneal it behind them, these processes drive torsional changes that alter

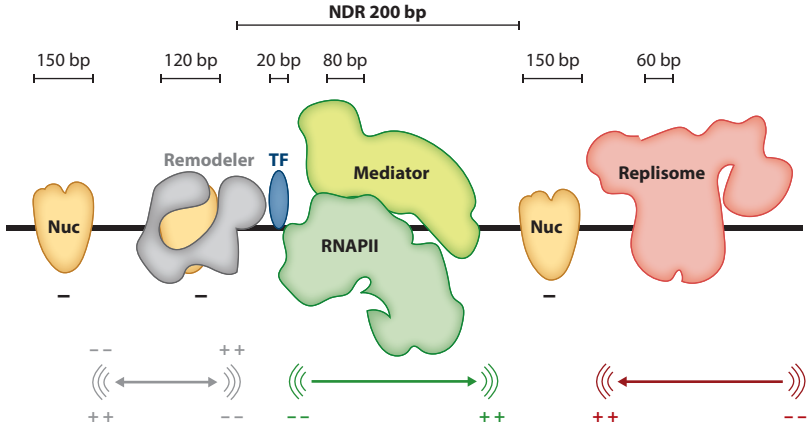

Figure 1

Chromatin proteins to scale. Protein complexes are sketched from 3D structures, and the segment of DNA that each protects is indicated. (*Left* to *right*) Nucleosome (wrapping 150 bp, *yellow*), nucleosome with chromatin remodeler (1) (120-bp footprint, *yellow/gray*), transcription factor (~20-bp footprint, *dark blue*), RNAPII and Mediator (2) (80-bp footprint, *green*), and a replisome (3) (60-bp footprint, *red*). An NDR is usually found at promoters of active genes. The wrapping of DNA around a nucleosome causes the DNA to be negatively supercoiled, indicated by a black minus sign below the nucleosomes. The movement of remodelers, RNAPII, and the replisome propagates positive supercoiling in front of these complexes and negative supercoiling behind them, indicated by red, green, and gray plus and minus signs for supercoiling and arrows for the direction of movement of these complexes (4). Lengths of DNA are not drawn to scale, as some structures, like nucleosomes, wrap DNA. Abbreviations: NDR, nucleosome-depleted region; Nuc, nucleosome; RNAPII, RNA polymerase II; TF, transcription factor.

factor binding and chromatin packaging. Thus, a principal requirement for genome function in eukaryotes is to manipulate the antagonism between packaging, exposure, and torsion of DNA. This entails cycles of assembly and disassembly of both chromatin and chromatin machines in living cells, and our understanding of these dynamics is informed by high-resolution structures, methods that map in vivo chromatin dynamics, and detailed modeling of assembly pathways. In this review, we focus particularly on the spatial and temporal restrictions that nucleosome dynamics impose on genome function and regulation.

2. CHROMATIN PROTEIN DYNAMICS

Binding of RNA polymerases, transcription factors (TFs), and chromatin remodeling enzymes to DNA are all dynamic. These proteins bind and dissociate continually, and a mechanistic understanding of chromatin function in living cells must take these dynamics into account. Studies of the dynamics of the transcriptional machinery in living cells have uncovered five surprises. First, TF binding at regulatory elements is fast, with residence times of seconds (5). While this is affected by the concentrations of TFs in cells and their specific binding kinetics, most transcriptional regulatory elements are only rarely engaged with *trans*-acting protein (6). Second, most genes are rarely transcribed in cells. In budding yeast, for example, approximately 170 genes account for half of the polyadenylated mRNA transcripts in a cell [calculated using 4-thiouracil labeled RNA-sequencing data (7)], and the average gene is transcribed on the order of once every few minutes to hours (8). This is especially true of genes for determinative TFs which are expressed at extremely low levels, in spite of the requirement that they be reliably expressed to maintain cell fates. Third, long-range enhancer–promoter contacts are rare and short lived (9). Enhancer function is conceptualized as acting by stabilizing large protein complexes at promoters, but it remains unclear how rare, transient contacts between enhancers and promoters affect transcriptional output. Fourth, most chromatin-binding events fail to be productive. For example, only 10% of RNA polymerase II (RNAPII) molecules that load at a promoter successfully initiate transcription, and only 10% of those initiation events successfully convert to elongation and transcribe a gene (10). Finally, transcription sputters, i.e., active promoters release sporadic bursts of multiple initiating RNAPII molecules (the number is referred to as the burst size), and these bursts occur only intermittently (burst frequency) (11). While some of these behaviors are inherent to the dynamics of factors binding to DNA, their dynamics are amplified by chromatin packaging. Thus, functional mechanisms must accommodate the sporadic nature of factor-binding events and integrate them with nucleosome dynamics within living cells.

3. DYNAMICS OF NUCLEOSOME POSITIONING

The bulk of the genome in most eukaryotes consists of intergenic sequence and is thus relatively quiescent (12). But, once every cell cycle, chromatin is completely disrupted as the DNA double helix is denatured and fed through the narrow pores of the replication machinery. Doubling of DNA necessitates doubling the histones and chromatin proteins that package that DNA (13), and therefore, chromatin duplication is coupled to DNA replication to efficiently rebuild chromatin in the wake of replication forks (**Figure 2**). Rebuilding chromatin is accomplished by the transfer of old histones from the parental chromatid to the two daughter chromatids, and full packaging of daughter chromatids is completed by the deposition of new histones (14).

Classic work established the patterns and rates of chromatin rebuilding behind the replication fork. These patterns are a direct consequence of the structure of the nucleosome. The nucleosome contains a tetramer subunit of $(H3{\bullet}H4)_2$ histones sandwiched between two H2A•H2B dimers, with ~146 bp of DNA in 1.65 left-handed wraps around this octamer (18). Arginine

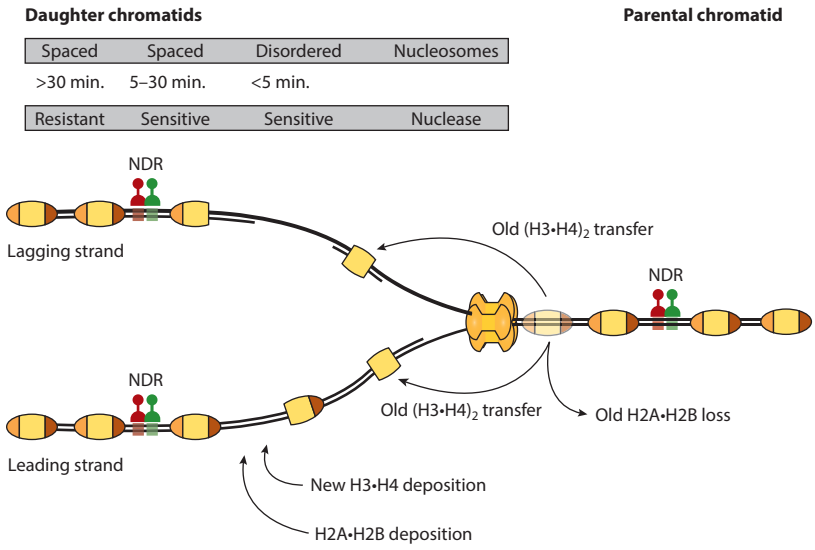

Figure 2

Chromatin duplication after DNA replication. In this schematic of chromatin replication, the replication fork is shown moving to the right; thus, the parental chromatid is to the right of the fork, and newly replicated daughter chromatids are to the left of the fork. Newly replicated chromatids remain nuclease sensitive for up to 30 minutes postreplication (15), and the deposited nucleosomes get ordered by remodelers 5–30 minutes postreplication (16), as seen in the map of time postreplication (shown above the daughter chromatids). Similarly, transcription factors start rebinding at most sites ~30 minutes postreplication in metazoans, creating NDRs (17). Abbreviations: NDR, nucleosome-depleted region.

residues from histones form hydrogen bonds with the DNA phosphate backbone to act as sprockets, which, together with electrostatic interactions between the basic surface of the histone octamer and acidic DNA, stabilize the wrapping of nucleosomal DNA (**Figure 3**). Together, the first H2A•H2B dimer organizes ~30 bp of DNA, the tetramer organizes ~60 bp, and the second H2A•H2B dimer organizes another 30 bp (two additional contacts between DNA and each H3 histone organize another 13 bp at the entry and exit sites of the nucleosome). As a replication fork approaches a parental nucleosome and denatures the DNA, a wave of positive supercoiling in front of the fork destabilizes the nucleosome by initiating unwrapping of the negatively supercoiled DNA around the histone octamer to ease fork progression. At first a H2A•H2B dimer is released, then the $(H3•H4)_2$ tetramer and the second dimer dissociate. The tetramer is captured by histone chaperones that accompany the replication machinery, including a histone-binding domain of the MCM helicase (19, 20) and subunits of DNA polymerase epsilon (21) for transfer to daughter strands, while the H2A•H2B dimers are released to the nucleoplasm (22). These two modes for nucleosome subunits are referred to as distributive segregation of $(H3•H4)_2$ tetramers and dispersive segregation of H2A•H2B dimers. Behind the replication fork, dimers are added to transferred parental tetramers to complete nucleosomes. This is sufficient only to partially package each daughter chromatid, and new nucleosomes are assembled in the gaps via the deposition of H3•H4 histones by the CAF1 chaperone (23). These new nucleosomes are completed by the addition of H2A•H2B dimers (**Figure 2**).

Transfer of histones and new histone deposition occurs on the order of seconds (28, 29), such that only ~250 bp of DNA is depleted of histones behind a replication fork. However, hundreds of kilobases of chromatin behind a replication fork remain nuclease sensitive for up to 30 minutes

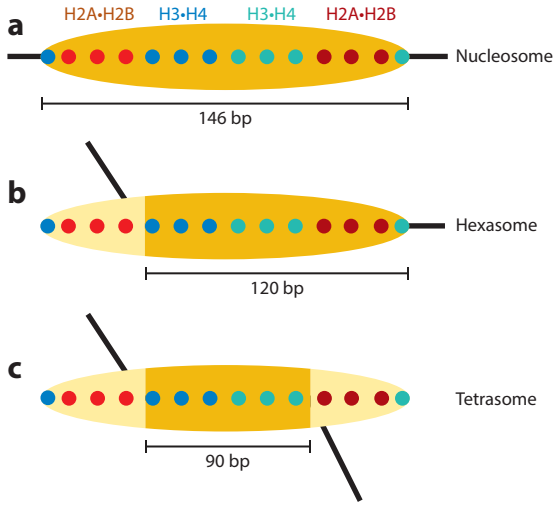

Figure 3

DNA wrapping and the subunit structure of the nucleosome. (*a*) Sketch of the length of DNA protected by a histone octamer with the arginine–phosphate contacts [sprockets (24)]. (*b*) Asymmetric unwrapping by processive helicases and polymerases would lead to progressive loss of the arginine contacts (25). (*c*) $(H3 \bullet H4)_2$ tetramers are minimal units deposited on newly replicated DNA (26, 27), engaging the central arginine sprockets.

after fork passage (30), during which time nucleosomes jostle for more stable positioning, and other chromatin proteins including H1 histones are added to further package the daughter chromatids (**Figure 2**). While these rates were calculated for newly replicated chromatin, similar rates should presumably apply for nucleosome assembly, repositioning, and adding chromatin packaging proteins after transcription and chromatin remodeling (**Figure 4**).

Genome-wide profiling of chromatin maturation now provides a more detailed picture of the variation in chromatin duplication across a genome. The rates of reestablishing chromatin structure after DNA replication vary at genomic locations and are linked to the functional properties of these sites. The transcriptional starts of genes, enhancers, and other regulatory elements are usually small segments dispersed through the vast expanse of bulk chromatin. For example, active promoters span ~200–300 bp of exposed DNA, referred to as a nucleosome-depleted region (NDR), where TFs and interacting proteins bind. The NDRs of active promoters are maintained by regulating the position and stability of local nucleosomes (31). But, since chromatin is completely disrupted by DNA replication, promoter nucleosome positioning and NDRs must be reestablished in the wake of the replication fork.

The size of a regulatory element affects its propensity to undergo nucleosomal packaging: Elements <200 bp in length cannot be wrapped into a nucleosome, and thus short promoter regions can be cleared by positioning their flanking nucleosomes. A single TF bound at a promoter can be sufficient to position these flanking nucleosomes and thereby maintain an NDR (32). In contrast, regulatory elements longer than 200 bp are long enough to wrap a nucleosome and therefore require active clearance of that sequence. This is accomplished by multiple DNA-binding proteins and the activity of chromatin remodelers (33).

In budding yeast cells, reestablishment of promoter NDRs occurs within minutes and is driven by the rapid rebinding of ubiquitous TFs like Abf1 and Reb1 behind the replication fork (34). In metazoans, the dynamics of reestablishing chromatin structure at many regulatory elements and

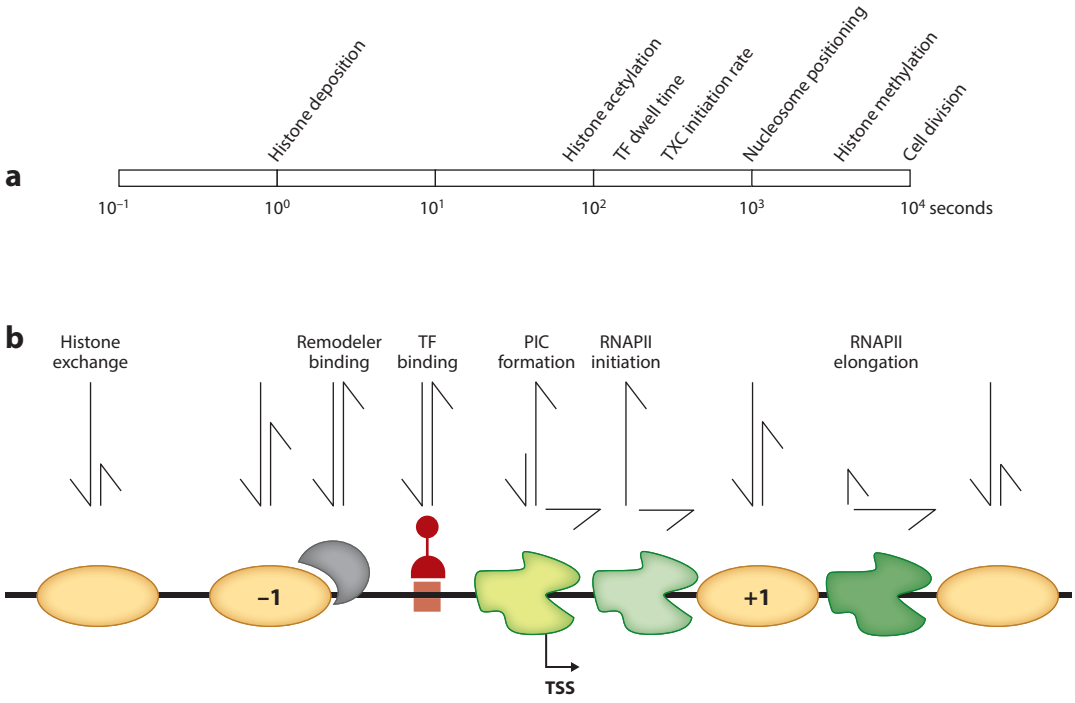

Figure 4

The timescales of chromatin dynamics. (*a*) Time in seconds plotted on a log scale, annotated with chromatin processes that occur at various timescales. (*b*) Schematic of the dynamics of chromatin proteins on DNA with length of arrows depicting relative rates. Nucleosome assembly is efficient due to chaperones and remodelers. Nucleosome disassembly is fast at regulatory sites compared to nonregulatory sites to facilitate TF binding. Rates of RNA polymerase successfully forming PICs and elongating are much lower relative to the off rates, based on observations that only a small fraction of polymerases successfully initiate and elongate (10). Abbreviations: PIC, preinitiation complex; RNAPII, RNA polymerase II; TF, transcription factor; TXC, transcription complex; TSS, transcription start site.

at the promoters for housekeeping genes are similarly rapid (17). These elements also use TF binding to quickly reestablish their chromatin structure at rates faster than those seen for bulk chromatin.

In contrast, the reestablishment of the chromatin structure of developmental promoters and enhancers is slow (17, 35) and is obscured by poorly positioned nucleosomes for more than an hour after replication. This delay implies that TFs take a substantial amount of time to rebind these elements after replication. This delay may allow for fine-tuning of gene expression. Furthermore, promoters that rely on tissue-specific TFs to position flanking nucleosomes will effectively limit NDR formation to cells expressing those TFs.

Changes in chromatin structure are most dramatic immediately after DNA replication. While comparing chromatin at different stages of the cell cycle gives the impression that nucleosomes are static outside of S phase, all nucleosomes are thermally dynamic, with DNA continually releasing from and rebinding the histone octamer. These dynamics are more extreme near regulatory elements and are crucial for maintaining the precise features of regulatory chromatin structure (36). Three mechanisms modulate nucleosome positioning in vivo, and each is a consequence of the structure of the nucleosome. First, some DNA sequences bend poorly around histone octamers and can intrinsically destabilize nucleosomes (37). However, most natural eukaryotic sequences

do not intrinsically position nucleosomes, and overall, this is a minor contribution in genomes. Second, opportunistic binding of sequence-specific factors can trap transiently exposed DNA and block wrapping of DNA into a nucleosome (38). Finally, translational sliding by chromatin-remodeling enzymes can expose DNA and drive nucleosomes to specific positions on DNA (38). Counterintuitively, the most well-defined features of genomes result from the most dynamic nucleosomes.

4. SUBNUCLEOSOMAL DYNAMICS

Nucleosome dynamics also have structural consequences for the histone octamer itself. When DNA is peeled off a nucleosome, contacts with an H2A•H2B dimer of the octamer are broken (39). Eviction of this dimer leaves a histone hexasome on the DNA. Such partial nucleosomes are present around active promoters and regulatory elements and contribute to the overall accessibility of DNA at these elements (25). Indeed, the genome of budding yeast, which is much more active than metazoan genomes, is predominantly packaged with subnucleosomal particles, providing an overall heightened level of genome accessibility (40, 41). Further peeling of the DNA past the H2A•H2B dimer into the tetramer destabilizes the entire octamer, leading to eviction. Thus, sites such as NDRs with high nucleosome mobility are also sites of high histone eviction (36, 42, 43).

Subnucleosomal dynamics also allow the specialization of chromatin regions with alternative histones. All eukaryotic genomes encode variant histones in addition to the core histone repertoire. Delivering variants to specific sites in genomes relies on the eviction of old histones at dynamic chromatin regions. For example, the metazoan H3.3 histone variant is deposited to fill gaps in chromatin after nucleosomes have been evicted at active promoters and regulatory elements (44–46). Localized deposition of the H3.3 variant is enhanced by specialized histone chaperones and deposition factors that further refine its genomic distribution (47). Similarly, enrichment of H2AZ variants in chromatin is driven by nucleosome destabilization, histone eviction, and recruitment of variant-specific chaperones and deposition complexes (48). The incorporation of H2AZ variant histones also alters RNA polymerase kinetics at promoters (49) and during elongation through nucleosomes (48), possibly by modulating DNA exposure dynamics.

5. ATP-DEPENDENT CHROMATIN REMODELERS DRIVE NUCLEOSOME DYNAMICS

Given the importance of DNA exposure rates for TF binding and RNA polymerase elongation, cells devote machinery to modulating nucleosome dynamics. Multiple SNF2-type ATP-dependent chromatin remodelers bind and mobilize nucleosomes (50). Structural studies of remodelers engaged with nucleosomes show that these motors break DNA–histone contacts, peel DNA off histone octamers, and propagate a wave of distortion around a nucleosome to move it to a new position (51). Remodelers are targeted to sites of chromatin accessibility (52) and act to either push or pull flanking nucleosomes (53). The timescales of remodeler action have been interrogated by acute chemical inhibition of the SWI/SNF remodeler Brg1 in mammalian cells (36, 43). Promoters are invaded by nucleosomes and lose TF binding within 10 minutes of Brg1 inhibition and then regain their chromatin structure within minutes of washing out the Brg1 inhibitor. Thus, the precise positioning of promoter chromatin architecture is achieved by increasing the dynamics of nucleosomes, such that NDRs and spacing are maintained. Chromatin remodelers may also alter the arrangement of nucleosomes. For example, the ISWI-, CHD-, and INO80-family remodeler enzymes have protein domains that act as rulers to fix the linker DNA length between nucleosomes, thereby altering the spacing in arrays (54). The functional outcomes of remodeling depend on which DNA sequences are exposed and which additional DNA-binding proteins bind

at remodeled regulatory elements. For example, in *Drosophila*, the ubiquitous TF GAF interacts with both Brahma and ISWI chromatin remodelers (55–58), which clear nucleosomes from regulatory elements. This potentiates the binding of additional TFs at some elements and the binding of silencing factors at others.

6. TRANSCRIPTION FACTOR BINDING TO DYNAMIC NUCLEOSOMES

The dynamics of nucleosome positioning and subnucleosomal structures are critical for TF binding in vivo. Wrapping double-helical DNA around the histone octamer means that the translational positions of a histone octamer are coupled to the rotational positioning of sequences around the nucleosome (59). Thus, translational movement of a nucleosome both exposes previously wrapped DNA at the edge and rotates the DNA on the histone octamer; this rotation also exposes sequences that were facing the histone octamer every 5 bp. Both DNA exposure at nucleosome edges and rotating sequences on the surface of the nucleosome modulate factor binding.

Due to the wrapping of DNA on the histone surface, only stretches of ~5 bp of a given DNA strand are exposed to the aqueous environment (59). The majority of TFs bind an extended DNA motif and so bind exposed DNA adjacent to the nucleosome (60). While nucleosomes limit binding by these factors, in vivo nucleosomes occupy an ensemble of positions (61, 62). Thus, in some cells, a DNA sequence may be accessible for binding, while in other cells, it is occluded. Transient site exposure by peeling also reduces the nucleosomal barrier to factor binding (63). Similarly, rotational dynamics of nucleosomal DNA allow binding by nucleosome-binding TFs (64, 65). Not only do nucleosome dynamics promote TF binding, but factor binding also alters nucleosome dynamics. Once a TF binds exposed DNA it prevents rewrapping of the DNA around a histone octamer (63), and this is a major contributor to maintaining NDRs at active regulatory elements.

TF binding can also drive dramatic changes in nucleosome structure. A small number of TFs bind short motifs on wrapped nucleosomal DNA and are thus modulated by the rotational positioning of motifs on the nucleosome surface. Nucleosome-binding factors such as Sox2 distort the DNA they are bound to, destabilizing a nucleosome (66, 67). However, nucleosomal binding is transient, as destabilizing the nucleosome exposes motifs for direct factor binding. Indeed, multiple nucleosome-binding factors appear to first bind in a nucleosomal mode and then switch to binding in an exposed DNA mode, apparently as more extended motifs are exposed (68).

How nucleosome dynamics and chromatin protein dynamics are integrated in living cells is now being revealed by single-molecule footprinting (6, 69–72). Single-molecule footprinting of chromatin in vivo detects bound factors and nucleosome positions across a DNA fiber, and these studies have resulted in surprising observations. First, nucleosome positions and spacing are heterogeneous between chromatids in different cells, implying constant widespread chromatin remodeling (71). These dynamics provide TFs the opportunity to bind transiently exposed DNA (38). Thus, nucleosomal binding by TFs may rarely be needed, as TFs can exploit transient DNA exposure in a constantly shifting nucleosome landscape. Second, factor-binding sites spend a substantial fraction of time neither bound by a TF nor occluded by nucleosomes but rather in an empty state with no factor and no nucleosome (6). This predicts short binding times for TFs, which is corroborated by single-molecule imaging studies (73). Third, many detectable TF-binding events are actually cobinding events with more than one factor at a regulatory element (6, 70, 72). Factor cobinding may efficiently displace nucleosomes and may be one function of juxtaposing multiple factor-binding sites within *cis* regulatory elements (74, 75). Together these single-molecule results suggest constant TF and nucleosome dynamics.

7. NUCLEOSOME DYNAMICS DURING TRANSCRIPTION

What about transcriptional elongation? Just like during replication, a polymerase that denatures and copies a DNA strand displaces histone octamers as it progresses. The eviction of histones in transcribed gene bodies is minor: only ~5% of that observed in regulatory elements (44, 76). This is in part because elements such as promoters are undergoing continuous remodeling, while nucleosomes in gene bodies are only momentarily disrupted upon occasional transcription. Additionally, proteins with histone chaperone activity accompany RNAPII, and these serve to transfer histones from in front of RNAPII back onto DNA in the wake of the polymerase (77). This transfer is assisted by topological changes driven by RNAPII progression. Denaturing the DNA strands propagates a bow wave of positive supercoiling ahead of the polymerase, promoting the disassembly of nucleosomes, while in the wake of RNAPII, negative supercoiling promotes rewrapping histone octamers into nucleosomes (78).

8. CHROMATIN DYNAMICS BEYOND THE NUCLEOSOME

While the largest inhibitory effects on DNA factor binding come from histones occluding DNA sequences, chromatin must be further compacted to fit into the nucleus. The higher-order organization of chromatin in the nucleus has been detailed at increasing resolution by both imaging and sequencing-based methods. Nucleosome arrays are partitioned into loops that are anchored by chromatin complexes, including boundary factors and cohesins, and these loops are loosely associated into active and inactive spatial compartments within the nucleus (79, 80). These higher-order structures form and dissolve, and recent experiments indicate that both loops and compartments reflect transient and relatively infrequent contacts between chromatin regions (81–83). How might large-scale transient interactions have effects on gene regulation? Some aspects of higher-order organization may limit or promote nucleosome dynamics. For example, steric effects between nucleosomes in an array limit translational repositioning and thereby reduce the exposure of sequences buried in a nucleosome. Steric effects should be most prominent in compacted nucleosome arrays, and thus, heterochromatin and inactive compartments may have relatively weak effects that accumulate over arrays. Alternatively, the promoter–enhancer loops may appear transient, as they might precede gene activation and become lost as a consequence of transcription (84).

Packaging DNA into the confines of a cell or a virion is a problem that confronts all forms of DNA-based life. In eukaryotes, DNA packaging is only partially solved by nucleosomes, which achieve a packing ratio of 6:1, whereas the mitotic chromosome packing ratio is ~8,000:1. Topoisomerases are ancient machines for packaging, having evolved prior to the emergence of the eukaryotic nucleus. Therefore, genome packaging must be distinct from nucleosome-based gene regulation. Each nucleosome accounts for one negative superhelical turn, and so complete removal or assembly of a nucleosome requires a single net swiveling (by topoisomerase type I) or DNA pass-through (by topoisomerase type II) event to locally relieve torsion and prevent supercoiling-driven displacement at a distance. Although the transient relief of torsion by topoisomerases is essential for normal gene expression, the possibility that modulation of torsion has also evolved roles in gene regulation (85–88) is an attractive area of future research.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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