Review

What controls nucleosome positions?

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The DNA of eukaryotic genomes is wrapped in nucleosomes, which strongly distort and occlude the DNA from access to most DNA-binding proteins. An understanding of the mechanisms that control nucleosome positioning along the DNA is thus essential to understanding the binding and action of proteins that carry out essential genetic functions. New genome-wide data on *in vivo* and *in vitro* nucleosome positioning greatly advance our understanding of several factors that can influence nucleosome positioning, including DNA sequence preferences, DNA methylation, histone variants and post-translational modifications, higher order chromatin structure, and the actions of transcription factors, chromatin remodelers and other DNA-binding proteins. We discuss how these factors function and ways in which they might be integrated into a unified framework that accounts for both the preservation of nucleosome positioning and the dynamic nucleosome repositioning that occur across biological conditions, cell types, developmental processes and disease.

DNA *in vivo* is highly compacted into chromatin

Eukaryotic genomic DNA is compacted through multiple steps into a protein–DNA complex known as chromatin. The first level of compaction involves wrapping the long genomic DNA molecules into arrays of particles called nucleosomes, each containing a 147-bp long stretch of DNA that is sharply bent and tightly wrapped in nearly two superhelical turns around an octameric core of 'histone' proteins [1]. Any DNA sequence can be wrapped into a nucleosome; however, homeostatic histone concentrations ensure that only ~75–90% of the DNA is wrapped in nucleosomes [2], with consecutive nucleosomes typically separated by ~20–50 bp of unwrapped 'linker' DNA. Thus, the limiting number of nucleosomes creates competition among different regions of genomic DNA.

Although other aspects of chromatin biology, especially the many post-translational modifications (PTMs) present on histones, have received the most attention, the detailed positions of nucleosomes along the DNA are also important. Wrapped nucleosomal DNA is much less accessible than linker DNA to the many proteins that act on naked DNA to carry out essential genetic functions. Nucleosomal DNA is sterically occluded by its close proximity both to the protein core of the nucleosome and to the other superhelical turn of DNA within the same nucleosome; binding of other proteins to nucleosomal DNA is further hindered by the sharp bending and altered helical twist of the nucleosomal DNA, which do not usually match the conformations favored by other DNA-binding proteins [1]. Thus, the genomic positions of nucleosomes strongly influence the ability of other proteins to bind to their own DNA target sites, enabling nucleosomes to function as both general repressors and activators of gene expression [3,4].

Indeed, in the simple organism *Saccharomyces cerevisiae*, the detailed locations of nucleosomes might be even more important than most histone PTMs: individual histone tail domains (where most histone PTMs occur) can be deleted altogether with only modest phenotypic consequences [5–7], whereas changes in nucleosome location with respect to regulatory protein DNA target sites influence protein occupancy by orders of magnitude *in vitro* [8,9] and lead to large, near-binary transcriptional and replication responses *in vivo* [10–12].

To better understand and predict when and where along the genome other DNA-binding proteins will act, we must understand where nucleosomes are located, and the principles that govern these locations. A series of rapid technical advances, first involving high resolution tiling microarrays [13–15] and, most recently, several different DNA sequencing technologies [16–26], have provided genome-wide maps of nucleosome locations for many different organisms and cell types.

As a result of this recent progress, we now recognize several different factors that can influence nucleosome positioning *in vivo*, including DNA sequence preferences of nucleosomes themselves, DNA methylation, histone variants and PTMs, higher order chromatin structure, and the actions of chromatin remodelers and DNA-binding proteins. We first review what is now understood about each of these influences on nucleosome positioning. We then discuss how these many factors can be integrated into a unified framework that might explain how changes in cell state or development can influence nucleosome positions and, conversely, how nucleosome positions influence cell state and development.

Nucleosomal DNA sequence preferences

DNA sequence preferences comprise the most studied factor that influences nucleosome positioning. Nucleosomes have at least a 5000-fold range of affinities for differing DNA sequences [27–29]. The large sequence specificities of most DNA-binding proteins result from direct interactions between specific functional groups on the protein- and base-specific atoms on the DNA. Nucleosomes, however, form few base-specific contacts between histones and DNA. Instead,

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Box 1. Nucleosome positioning and nucleosome occupancy

We define nucleosome positioning at a given base pair as the probability that a nucleosome starts at that base pair. At equilibrium, this probability is the same for a single cell when observed (averaged) over long time, or for a large population of cells observed at any instant in time. We define nucleosome occupancy at a given base pair to be the total probability with which that base pair is covered by any of the nucleosomes that could potentially cover it. Given that nucleosomes occupy 147 bp, 147 different nucleosomes potentially exist that could cover a given base pair. Because nucleosomes occupy space, two different nucleosomes cannot simultaneously cover any one base pair in any given cell. The occupancy at base pair *i* is thus the sum of the probabilities of the all of the (mutually exclusive) nucleosomes starting from base pairs *i–146* to base pair *i*. Occupancies, like probabilities, vary between 0 and 1.

Ultimately, one wishes to experimentally measure the exact distribution of nucleosome positions (the probability of a nucleosome starting at each base pair, genome-wide). However, when data are sparse and/or noisy (as in all presently available genome-wide nucleosome maps), occupancy offers some advantages. First, nucleosome occupancy as defined earlier is precisely equal to a 147-bp moving average of the nucleosome positioning probabilities (for positioning as defined above). Such averaging can help to provide more robust conclusions. Second, whether, or how much, a DNA-binding protein seeking to bind over a given base pair must compete with nucleosomes depends in part on the nucleosome occupancy over that base pair. TF-binding sites, which have intrinsically low nucleosome occupancy, will on average be more easily occupied by the cognate factor for a given concentration of that factor than will be binding sites having intrinsically high nucleosome occupancy.

Some investigators, however, prefer a hybrid definition of positioning, reflecting a particular compromise between exact positioning and occupancy. One group, for example, counts the number of measured nucleosome centers falling within a 23-bp window (centered on the base pair in question plus 11 bp on each side) divided by the total number of measured nucleosome centers within a 301-bp window (the base pair in question plus 150 bp – approximately one nucleosome length – on each side) [19].

nucleosome preferences probably stem from the sequence-dependent mechanics of the wrapped DNA itself [30]. DNA sequences differ in their abilities to bend and alter their helical twist. The exceptionally sharp DNA bending enforced by the nucleosome exaggerates what might otherwise have been only modest differences in the structural and mechanical properties of different DNA sequences, thereby conferring a wide range of sequence-dependent affinities for nucleosome formation.

Early studies suggested that intrinsic DNA sequence preferences could have a role in nucleosome positioning *in vivo* [29,31–33]. Subsequent analyses showed that *in vitro* nucleosome preferences often mirror *in vivo* locations [27,34] and that nucleosome occupancy *in vivo* could be predicted based on the genomic DNA sequence alone [26,35–38].

A recent study in *S. cerevisiae* showed directly that the intrinsic DNA sequence preferences of nucleosomes have a dominant role in nucleosome organization *in vivo* [25]. Nucleosomes were reconstituted *in vitro,* in a system comprising only purified histones (from a foreign organism, chicken) and yeast genomic DNA, followed by genome-wide mapping of the reconstituted nucleosomes. The resulting distribution of nucleosome occupancy (Box 1) was highly similar to that obtained *in vivo* (correlation = 0.74 across all base pairs), and a sequence-based model of nucleosome positioning derived using only these *in vitro* nucleosome data was strongly predictive of *in vivo* nucleosome occupancy (correlation = 0.75 across all base pairs, accuracy of 92% in distinguishing high from low nucleosome occupancy regions across the entire genome).

Importantly, genomic regions that are crucial for transcriptional regulation, including many promoters and transcription factor (TF)-binding sites, exhibit nucleosome depletion *in vitro*, suggesting that nucleosome depletion at these sites *in vivo* is partly encoded in the genome through nucleosome sequence preferences.

DNA sequence preferences of nucleosomes comprise two broad types, independent of the organism from which the nucleosomes are obtained: (i) preferences for certain sequence motifs to occur at particular locations within the nucleosome; and (ii) preferences for other motifs to not occur anywhere inside a nucleosome. The position-dependent motifs were initially characterized as particular dinucleotides that tend to occur periodically throughout the nucleosome, with a ~10-bp periodicity. These dinucleotide preferences are now partially understood and arise owing to the near-circular wrapping of the nucleosomal DNA, which requires sharp bending every DNA helical repeat (~10 bp), when the sugar-phosphate backbone (and minor groove) of the DNA faces inwards towards the histone octamer, and again 5 bp away with opposite direction when the backbone faces outward [30,32]. Specific dinucleotides facilitate bends of each direction [39], helping to explain why particular dinucleotides are favored at particular locations in each helical turn inside the nucleosome [14,40]. In fact, these preferences for particular motifs at particular locations within the nucleosome apply not only to dinucleotides but to longer DNA motifs as well [19,29]. The ability of a given dinucleotide to deform in a particular way (as required for nucleosomal wrapping) should depend in part on the immediate neighbors of that dinucleotide – and in turn on their neighbors, and so on; however, these longer motifs remain poorly explored. Finally, histone arginine side chains that insert into the minor groove every DNA helical turn could potentially provide additional ~10-bp periodic base-specificity, although these arginines are primarily bonded to the DNA backbone or to other protein groups [1].

The other major class of nucleosome DNA sequence preference is a negative preference: certain motifs are disfavored from being located anywhere inside a nucleosome. These include many different 5-mers [25,26] and, especially, long tracts (e.g. 10–20 bp or more) comprising primarily As on one strand and Ts on the other [13–16,26]. The molecular basis of these negative preferences is only partly understood. All that is known with certainty is that, for some reason, these particular DNA sequences resist the structural distortions that are required for nucleosome formation. Poly(dA:dT) elements have long been suspected to be especially stiff and, thus, especially resistant to the sharp bending required by the nucleosome; however, direct tests have failed to detect such enhanced stiffness and its detailed origin (if even true) remains a matter of debate

[41]. Nevertheless, poly(dA:dT) elements, by virtue of their nucleosome-excluding properties, are major determinants of the global *in vivo* nucleosome organization, generating a zone of strong nucleosome depletion centered over the poly(dA:dT) elements and contributing, together with other DNA-binding proteins, to the creation of a concomitant long range ordering of nucleosomes to either side [41,42].

A statistical model of DNA-sequence-dependent nucleosome positioning captures many of these nucleosome DNA sequence preferences, using only data from *in vitro* nucleosome reconstitution on yeast genomic DNA [25]. The model explicitly represents the position-dependent preferences for each dinucleotide at each position throughout the nucleosome, together with the preferences for each 5-mer to be located inside the nucleosome (or not). Despite being trained only on *in vitro* yeast nucleosome data, the resulting model discriminates nucleosome-enriched regions from nucleosome-depleted regions in yeast *in vivo* with high accuracy, and is significantly predictive of the *in vivo* nucleosome occupancy in *Caenorhabditis elegans*. In summary, intrinsic nucleosome DNA sequence preferences, based on DNA-sequence-dependent structures and mechanics, are major, and perhaps universal, determinants of nucleosome organization *in vivo*.

DNA methylation

In higher eukaryotes, cytosine DNA methylation at CpG dinucleotides has an important role in regulating gene expression. Because methylation chemically modifies DNA, the methylation status of a DNA sequence could influence its flexibility and, thus, its affinity to the nucleosome. Several studies point to a strong link between CpG methylation and nucleosome positioning, but these effects might be indirect because methylation influences the binding of other factors, which could in turn trigger nucleosome repositioning. We review what is known about the direct influence of CpG methylation on nucleosome positions.

Essentially, all possible answers are present in the literature; however, a consensus is emerging that CpG methylation can decrease the ability of DNA to bend into the major groove at the methylated CpG step, and can thereby influence nucleosome positioning. Although early studies failed to detect effects, more recent studies showed that CpG methylation decreased the DNA bending flexibility [43] and an analysis by X-ray crystallography showed that CpG methylation disfavored the formation of bent structures ordinarily stabilized by the polycation spermine [44]. Increased steric bulk in the DNA major groove, resulting from the added methyl group, could limit the ability of the DNA to bend in a direction that requires compression of the major groove, such as occurs every ~10 bp on the nucleosome surface. Consistent with this possibility, some direct measurements of nucleosome affinity for methylated DNA show decreased affinity (and consequent nucleosome repositioning) [45] with results dependent upon the specific DNA sequence, and on the extent of CpG methylation. Thus, although the overall importance of DNA methylation as a direct determinant of nucleosome positioning *in vivo* remains poorly understood, the available evidence suggests that CpG methylation can directly influence DNA bendability and nucleosome positioning.

Histone variants and post-translational modifications

Beyond the DNA sequence itself, which obviously differs between distinct nucleosome positions, the histone proteins can also differ, either by PTM or by replacement with a histone variant. Such changes can influence nucleosome positions indirectly because many site-specific DNA-binding proteins can bind (directly or through other partners) specific modified or variant histones. Here, we consider whether the particular histone forms can also influence nucleosome positioning directly.

Histone PTMs occur primarily in the tail domains. Hyperacetylation or complete removal of the tail domains results in a small, but significant, increase in the accessibility [46,47] and stability [48] of nucleosomal DNA and in small (~20 bp) sequence-dependent changes in positions of some nucleosomes, although high-affinity sequences are not affected [49]. If hyperacetylation and entire tail removal represent lower and upper bounds on the magnitude of the effect of histone modifications, respectively, then histone modifications might have only a modest direct effect on nucleosome positioning. The biological implications of these effects remain unclear. We emphasize, however, that even if the direct effects of histone modifications on nucleosome positions are small, their indirect effects could be substantial, influencing protein–protein interactions and recruitment of ATP-dependent chromatin remodeling factors (Box 2).

Most core histone proteins have several variants with minor (e.g. H2B.1, H3.3) or major (e.g. H2A.Z, H2A.Bbd, centromere protein A [CENP-A]) differences in their amino acid sequences. These variants are associated with particular biological processes and exhibit specific expression patterns and localization to specific chromosomal regions. Nucleosomes containing histone variants might have altered bonds and interactions with DNA, which could manifest in changes in DNA sequence preferences and nucleosome positions. For example, CENP-A, which

Box 2. ATP-dependent chromatin remodeling factors

Cells contain diverse sets of large macromolecular machines, known as ATP-dependent chromatin-remodeling complexes [96], which use the energy of ATP hydrolysis to move nucleosomes to different locations along the DNA or to disassemble nucleosomes and remove them altogether [97,98]. These ATP-dependent remodeling factors seem to be recruited to specific chromatin regions through the prior actions of other site-specific DNA-binding factors [96].

The existence of these remodelers potentially challenges equilibrium models of nucleosome positioning. *In vitro*, these machines cannot only disassemble nucleosomes, but they can actively drive nucleosomes away from presumed equilibrium positions [99,100], even off the nucleosome positioning sequences that have the highest known affinities; and they can position nucleosomes in the center of the DNA between two neighboring nucleosomes regardless of the underlying DNA sequence.

Notably, however, the altered nucleosome positions adopted upon treatment with some remodeling complexes are essentially identical to the initially favored positions: only the relative occupancies at those detailed positions change [99]. Thus, equilibrium nucleosome positioning seems to be relevant even during remodeling activity. Indeed, such remodelers might actually function *in vivo* to facilitate the establishment of a nucleosome positioning equilibrium. This fundamentally important question requires further analysis.

replaces H3 at centromeric nucleosomes, contains an insertion of two amino acids in a loop involved in a DNA contact [50]; moreover, centromeric nucleosomes are thought to contain only one copy of each histone and wrap less than the canonical 147 bp [51]. Nucleosomes containing the H2A variant H2ABbd similarly wrap <147 bp [52]. It remains unknown whether the DNA sequence preferences of these variant-containing nucleosomes differ from those of generic nucleosomes. However, systematic genome-wide data reveal that the sequence preferences of H2A.Z-containing nucleosomes are unchanged [18].

Finally, we note that nucleosomes often contain an additional protein, the 'linker histone' H1 or its variants [53,54]. The presence of H1 in a nucleosome can subtly influence the DNA sequence preferences of the nucleosome [55,56], although major features including the ~10-bp periodic occurrences of key dinucleotides remain unchanged.

Higher order chromatin structure

In vivo, nucleosomes are present in long and dense (concentrated) 1D arrays, which are further organized in 3D space. The high density of nucleosomes along the DNA, which varies between organisms and even between cell types in the same organism during normal differentiation and cancer [57], leads to two important consequences for nucleosome positioning. At the simplest level, the nucleosome density imposes a trade-off between occupancy (coverage) and sequence specificity in nucleosome positioning: at low densities, nucleosomes will favor high affinity sites, whereas higher densities inevitably require nucleosomes to be positioned over lower affinity sites as well. Thus, changing nucleosome density imposes a tradeoff between occupancy and affinity. More importantly, the high density of nucleosomes along the DNA, together with steric exclusion, which prevents consecutive nucleosomes from overlapping in space, strongly influences nucleosome positioning over long distances, in a phenomenon known as statistical positioning [42]. Genomic regions that strongly bind a nucleosome or strongly exclude a nucleosome (such as the poly(dA:dT) elements that are often found near gene termini) act as 'boundaries', delineating a stretch of the nucleosome array [16,26]. The detailed positions of nucleosomes within this stretch can depend on exactly how many nucleosomes are present (i.e. on the nucleosome density) because nucleosomes readjust their locations to avoid steric overlap [42,58,59].

Another way in which higher order chromatin structure influences nucleosome positions is through repulsive and attractive interactions between nucleosomes that neighbor in 3D space. At the most basic level of such higher order organization, geometrically complex repulsive constraints imposed by steric exclusion might dictate the allowed lengths of the linker DNA between two consecutive nucleosomes [60,61]. Such conclusions, however, are based on viewing nucleosomes as hard sphere objects, whereas other experiments show that nucleosomes can change their conformation with finite energetic cost [62,63], a property that might enable all linker lengths to exist, although with differing intrinsic free energies and, thus, differing probabilities. Energetically important attractive nucleosome–nucleosome interactions capable of influencing nucleosome positions also exist [64,65], most probably mediated by one or more histone N-terminal tail domains [66] and dependent on the acetylation status of Lys16, the histone H4 N-terminal tail [67].

An important unanswered question, however, is which nucleosomes in a nucleosome array exhibit attractive or repulsive interactions. Depending on the structure of the chromatin fiber, differing pairs of nucleosomes will neighbor in 3D space and, thus, could generate favorable or unfavorable nucleosome–nucleosome contacts.

The higher order structure(s) of chromatin remain unresolved [68–70], but several lines of evidence indicate that preferred intrinsic structures might be encoded directly in the genome. Analyses of *in vitro* nucleosome maps of the chicken and ovine β-globin loci [27,71] reveal long range periodicities between nucleosomes, suggesting that higher order structural properties of the chromatin fiber are encoded in the genome. Similarly, analyses of nucleosome repeat lengths in several organisms [61,72] and dinucleosome sequences in yeast [73] and human [74] also suggest that linkers have preferred lengths, of the form $10n+k$ base pairs (where k varies between analyses), and that these length preferences are encoded in the DNA. Such encoded structures would influence which nucleosomes are intrinsically likely to neighbor in space and, thus, experience potential repulsive or attractive interactions.

Finally, the effect of the higher order nucleosome organization on nucleosome positioning might also depend on the role of the linker histone H1 in DNA packaging because H1 facilitates the folding of chromatin into ~30 nm fibers [75,76] and partial H1 depletion results in shorter linkers (higher nucleosome density, perhaps corresponding to changing average values of n if preferred linker lengths are indeed encoded as described earlier) and reduced chromatin compaction in *Drosophila melanogaster* and mouse [77,78]. Although it is clear that higher order chromatin structure can affect nucleosome positions, we are far from a quantitative understanding.

TFs

Nucleosome positions *in vivo* can be directly influenced by the actions of ATP-dependent chromatin remodeling factors (Box 2) and TFs. TFs can influence nucleosome positioning by competing with nucleosomes for access to DNA. Structural studies of protein–DNA complexes reveal that many site-specific DNA-binding proteins wrap around and occlude much of the surface of their DNA target sites or require a DNA conformation very different to that in nucleosomes. Such factors cannot occupy their DNA target sites when present inside a native-state nucleosome and, therefore, might compete with nucleosomes for target site occupancy. Consistent with this view, global measurements of *in vivo* nucleosome positions in yeast find that many of the bound TF sites reside in nucleosome-depleted linker regions [13,14,18]. Part of this nucleosome depletion over factor-binding sites is intrinsically encoded in the genome, through the DNA sequence preferences of the nucleosomes. However, this is clearly not the sole mechanism: several studies show that nucleosomes occupy TF sites and must be repositioned to enable TFs to access their

sites [79,80]. Indeed, a comparison of the nucleosome locations that are intrinsically encoded in the genome (the *in vitro* nucleosome map) with maps of actual nucleosome locations *in vivo* [25,26] reveals numerous locations at which *in vivo* nucleosome depletion is greater than that observed in the *in vitro* map, pointing to an important role for TFs in nucleosome depletion.

Thus, competition between TFs and nucleosomes for occupancy of factor DNA target sites is an important determinant of nucleosome positions *in vivo*. At each genomic region, the outcome of this competition probably depends on both the relative affinities of the nucleosomes and TFs to the underlying DNA and on their concentrations. The detailed mechanism for such competition is not known: it could result from site exposure, whereby the DNA is partially unwrapped from one end of the nucleosomes with some energetic cost, thus enabling TF access [9,81]; alternatively, TF binding to sites occupied by nucleosomes might require complete displacement or disassembly of the nucleosomes from the site [82], possibly catalysed by remodeling factors.

TF–nucleosome competition points to two types of synergy or cooperativity in the binding of TFs to nucleosomal DNA target sites. First, binding of multiple proximal TFs to DNA will be inherently cooperative, through the collaborative competition of TFs against nucleosomes: upon binding of one TF to DNA and removal of (or site exposure in) the interfering nucleosome, subsequent TFs will more easily bind the now nucleosome-free DNA. Such binding cooperativity is observed both *in vitro* [83,84] and *in vivo* [85,86] and occurs even between arbitrarily chosen pairs of unrelated TFs, suggesting that cooperativity is a result of competition against nucleosomes and not just of specific protein–protein interactions. This universal cooperativity mechanism between TFs has intriguing implications for the way in which combinatorial regulation might evolve in eukaryotes. For example, if an organism must evolve an AND gate between two arbitrary TFs, such that activation is achieved only if both TFs are bound to DNA, then such a transcriptional program could arise by co-evolving the two TFs to specifically bind each other or to bind a third mediator protein. By contrast, cooperativity through competition with nucleosomes offers an alternative and possibly simpler solution, because it only requires juxtaposing DNA-binding sites for the two TFs next to each other, such that they are contained within the same nucleosome and, therefore, cooperate automatically.

Competition between TFs and nucleosomes also makes possible a second kind of cooperativity or synergy, between a site-specific DNA-binding TF and a DNA sequence element that, on its own, disfavors nucleosome occupancy, for example, a poly(dA:dT) element. As described earlier, such DNA elements disfavor nucleosome occupancy not only over themselves, but also extending considerable distances into the adjacent DNA regions, including TF-binding sites. This intrinsic nucleosome depletion makes it easier, in a thermodynamic sense, for TFs to occupy sites that are linked in *cis* to such nucleosome-disfavoring DNA sequences, and the more-favorable binding by those TFs further depletes nucleosomes from the region. Such synergy or cooperativity might influence nucleosome positioning in the large class of poly(dA:dT) element-containing promoters *in vivo* [26].

Thus, a given genome contains regions in which the intrinsic nucleosome organization leaves TF-binding sites relatively nucleosome-free. In such regions, TFs should have negligible effects on nucleosome positioning. In other regions, TFs will both compete with nucleosomes and affect positioning, and the inherent cooperativity of this TF-nucleosome interplay might be exploited by organisms for evolving novel transcriptional programs. Nevertheless, we are still far from a quantitative understanding of these phenomena.

Putting it all together: an equilibrium model for dynamic nucleosome positioning

Several decades of chromatin studies collectively show that many nucleosomes change their positions between biological conditions, cell-cycle timing and cell types, whereas the positions of many other nucleosomes remain unchanged. When nucleosomes redistribute their positions in response to changes in signaling or development, the redistribution often requires the action of one or another ATP-dependent chromatin remodeling complex. Thus, one possibility is that specifically recruited remodeling factors drive the movement of nucleosomes to particular new locations. This, however, leaves open the question of how the remodeling complexes know where to move the nucleosomes.

Here, we suggest an alternative view under which much, and possibly all, of the changes and preservations of nucleosome positions can be understood with the five effectors of nucleosome positioning discussed earlier. We hypothesize that nucleosomes and TFs occupy DNA in thermodynamic equilibrium. Assumptions of equilibrium, although unproven, have been consistently valuable in quantitative analyses of gene regulation in prokaryotes [87] and eukaryotes [88,89]. We consider that, at any given moment, every cell has a particular (and measurable) constellation of transcription factors, histone variants, post-translationally modified histones and DNA methylation status. At thermodynamic equilibrium, these inputs, together with the rules by which the various inputs affect (and are affected by) nucleosome positions, dictate a particular probability distribution over all possible configurations of nucleosome positioning and occupancy and bound factor occupancy that will result (Figure 1).

Recent studies provide support for this view because computational predictions of nucleosome positions, based only on nucleosome DNA sequence preferences, can predict nucleosome occupancy *in vivo* with high accuracy [25]. Extending the quantitative modeling to encompass additional influences on nucleosome positioning will probably improve the agreement. Importantly, the need for chromatin remodeling complexes in nucleosome repositioning is not excluded by this view: on the contrary, remodelers might be required *in vivo* to enable nucleosomes to equilibrate their locations.

How can this view account for both changes of some nucleosome positions and constancy of others? The genome remains fixed across biological conditions, but the constellation of other inputs (e.g. TF and nucleosome

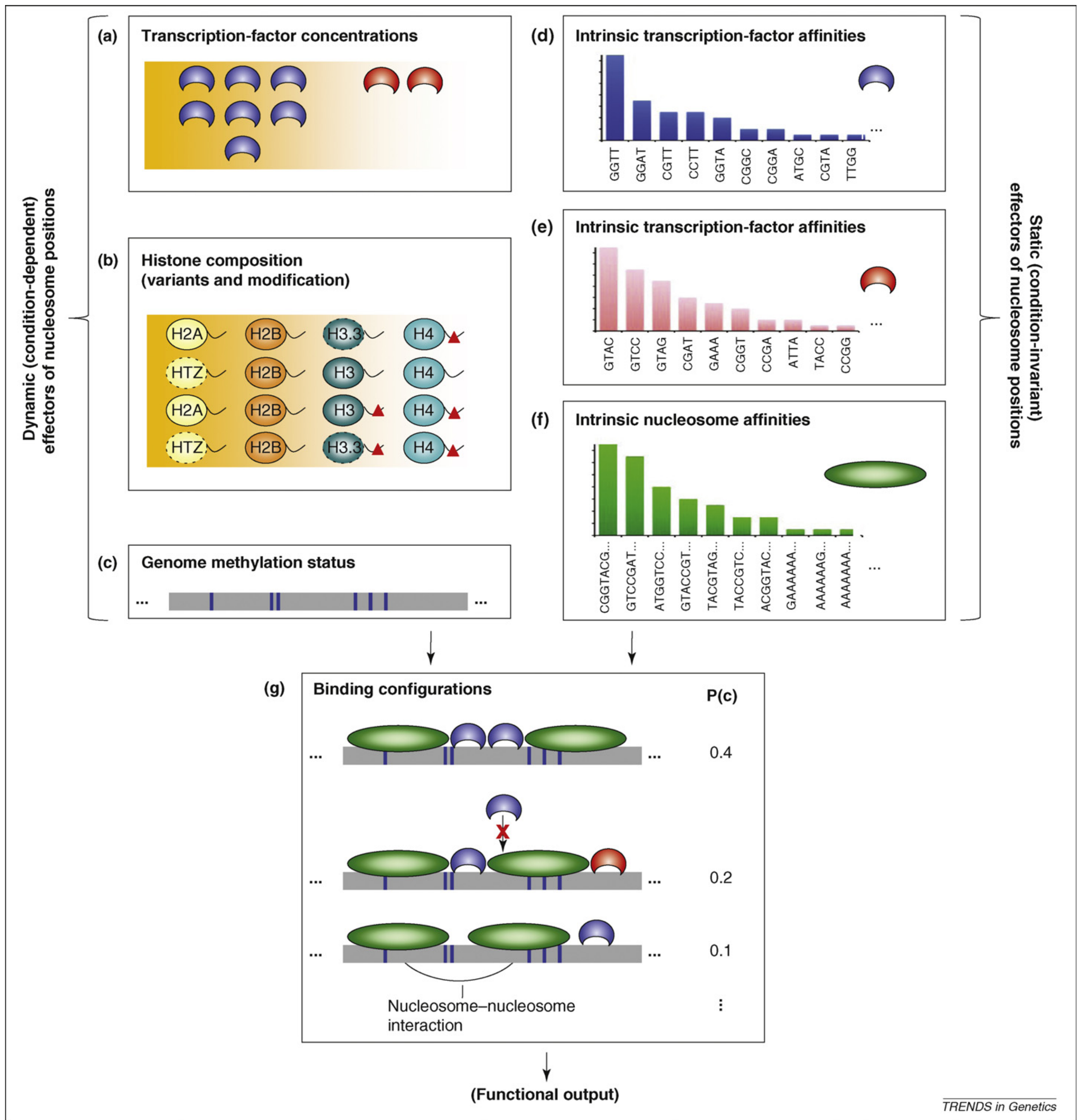

Figure 1. A unified framework for nucleosome positioning. Here, we present an illustration of our unified view, in which nucleosome positions are explained by the combined effect of both static, condition-invariant inputs such as nucleosome and TF sequence preferences **(d–f)**, and dynamic, condition-dependent inputs such as the concentrations of histones (not shown) and TFs, the composition of histones, and the methylation status of the genome **(a–c)**. Two different TFs are colored blue and red; different histones are colored yellow (H2A), orange (H2B) or turquoise (H3, H4); histone variants are outlined with a dashed line; PTMs are shown as red triangles; resulting nucleosomes are colored green; methylated CpGs are shown as blue lines through the grey DNA; and an occluded TF-binding site is indicated by a red X. Each input influences the positions of nucleosomes according to specific rules. The model that integrates these inputs assumes that nucleosomes equilibrate their positions, such that every possible configuration of nucleosomes and TFs on the DNA is sampled (g; shown is a small subset of the possible configurations). The probability that the system will be in any one configuration, P(c), is computed from the statistical weight of each configuration, which depends in turn on the concentrations of histones and input TFs and their affinities to the DNA sites they occupy in the configuration, taking into account the effects of the higher order chromatin structure of the configuration. The affinity of every nucleosome in a configuration is computed from its DNA sequence preferences, histone composition, and methylation status of the underlying DNA. Overlaps between two molecules in any one configuration are not allowed owing to steric hindrance and, thus, both nucleosome–nucleosome and nucleosome-factor competition is modeled. Finally, although our focus here is on the determinants of nucleosome organization, we note that the distribution of molecule-binding configurations will partly dictate the behavior of various functional outputs, including transcription, recombination, DNA repair and replication.

concentrations, DNA methylation, etc) varies, thereby potentially explaining the observed repositioning of some nucleosomes across conditions. However, other nucleosomes might resist repositioning, perhaps because nearby TF-binding sites have intrinsically low nucleosome occupancy, such that factors can bind or dissociate with no change in the locations of nearby nucleosomes. Alternatively, certain nucleosomes might be especially strongly positioned, such that TF-induced changes in nucleosome locations occur but are highly localized, perhaps simply leading to a gain or loss of a single nucleosome.

As another example, consider the case of a self-renewing stem cell. How are nucleosome positions re-established after the asymmetric cell division? Our view requires only that important factors maintain their concentrations (i.e. a similar constellation of inputs); consequently, a similar nucleosome organization would result. By contrast, other views must invoke specific and highly detailed machinery for actively placing nucleosomes back at their original positions following replication.

Can our view provide an appropriate level of description, or must we also consider dynamics – that is, the detailed pathways through which changing constellations of inputs generate changes in nucleosome positioning? A comparison to the genetic switch of the bacteriophage lambda is instructive. The gene expression patterns and epigenetic stability of both the lysogenic and lytic states of phage lambda can be separately understood by an equilibrium thermodynamic model [90]. The only aspect of the system that is not amenable to the equilibrium analysis is the dynamics of the state change itself, that is, the detailed process by which a lysogenic phage transitions to lytic growth. This is an intrinsically dynamic property of the system, which requires a detailed kinetic description. Understanding how the transition occurs is of interest in its own right, but it is not essential to understand the stability of either of the two states themselves.

There remain several grounds on which our equilibrium view can be questioned. ATP-dependent chromatin remodeling complexes could actively subvert equilibrium, but they need not, because equilibrium positioning seems to be relevant even during remodeling activity (Box 2). Other grounds on which to question our equilibrium model concern the detailed kinetics of regulatory protein binding and dissociation. Equilibrium requires that relevant regulatory proteins bind and dissociate rapidly relative to the transition of a bound RNA polymerase from a closed to open complex. However, in eukaryotes, the slow kinetics of *in vitro* TBP binding and dissociation [91], the existence of an ATP-dependent factor that specifically drives TBP off TATA boxes [92], and the frequent presence of stalled but poised RNA polymerase II at diverse gene promoters [93] all raise questions about the validity of equilibrium descriptions.

Nevertheless, as mentioned earlier, equilibrium models have proven highly successful in quantitative descriptions of gene regulation in prokaryotes and eukaryotes, and also for predicting *in vivo* nucleosome organization [25]. Because the equilibrium model is the simplest one, and yet successfully accounts for many aspects of nucleosome organization, we currently favor this view over alternative, more complex kinetic descriptions. We expect that continued development of this equilibrium model will lead to further understanding of the mechanisms underlying nucleosome positioning and to even better predictive power.

Concluding remarks and future perspectives

We have summarized the current understanding of the key factors that control nucleosome positioning, including factors that act at the level of single nucleosomes, factors that affect positioning through the higher order chromatin structure and factors that are extrinsic to both the DNA sequence and chromatin structure, namely the effects of chromatin remodelers and TFs. We then presented a view in which these rules, combined with the input constellation of TFs, histones and DNA methylation status of the genome, might be sufficient to explain how nucleosomes are positioned *in vivo*. This model assumes that nucleosome positions exist in a thermodynamic equilibrium, dictated only by the constellation of the input factors and the rules by which they affect nucleosome positions. Notably, although the rules are fixed, the inputs vary across biological conditions and, thus, this model can in principle also explain the dynamic repositioning of nucleosomes across different biological conditions.

Beyond the features that we discuss here, other factors, including the nuclear organization of DNA relative to nuclear compartments [94,95], will probably influence nucleosome positioning but little is currently known about such effects. However, as data addressing their effect on nucleosome positions become available, the modular nature of the equilibrium framework should make it easy to extend the model and integrate this information. Ultimately, as we accumulate more data and improve our understanding of the factors discussed earlier, and integrate new factors, it will be interesting to test whether the equilibrium framework can provide a quantitative and predictive understanding of chromatin structure across all eukaryotes and biological conditions, paving the way towards a true quantitative understanding of transcriptional control.

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