MODES OF TRANSCRIPTIONAL REGULATION

Determinants and dynamics of genome accessibility

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Abstract | In eukaryotes, all DNA-templated reactions occur in the context of chromatin. Nucleosome packaging inherently restricts DNA accessibility for regulatory proteins but also provides an opportunity to regulate DNA-based processes through modulating nucleosome positions and local chromatin structure. Recent advances in genome-scale methods are yielding increasingly detailed profiles of the genomic distribution of nucleosomes, their modifications and their modifiers. The picture now emerging is one in which the dynamic control of genome accessibility is governed by contributions from DNA sequence, ATP-dependent chromatin remodelling and nucleosome modifications. Here we discuss the interplay of these processes by reviewing our current understanding of how chromatin access contributes to the regulation of transcription, replication and repair.

Linker histones
Linker histones are not part of the nucleosomal core but, at least in the case of the linker histone H1, bind to DNA adjacent to the octamer.

Thermal motion
In the context of nucleosomes, *in vitro* experiments under physiological salt conditions revealed that higher temperatures, especially at 37 °C, promote short-range movement (that is, tens of base pairs) of nucleosomes in *cis*.

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Genome regulation occurs at the level of chromatin. The fundamental subunit of chromatin is the nucleosome core particle, which consists of DNA wrapped around a protein octamer composed of the four core histones (H3, H4, H2A and H2B). At the basic organizational level, nucleosomes are arranged into 'beads on a string', which confers a 5- to 10-fold compaction of the genomic template. In addition, metazoan chromatin contains linker histones, which bind in between nucleosomes and interact with core histones to form condensed fibres that are characterized by levels of compaction on the order of 50-fold and higher. The detailed organization of these higher-order structures is, however, less well understood[1]. Nucleosome packaging restricts protein binding and interferes with DNA-templated reactions. Local modulation of DNA accessibility thereby provides an opportunity to influence the fundamental processes of transcription, replication and repair. Indeed, chromatin structure is not static but subject to dynamic changes at every level of its hierarchy. Several determinants of DNA accessibility have been identified at the primary level of nucleosome arrays, and their interplay and function will be the main focus of this Review.

In vitro studies show that nucleosomes display substantial DNA sequence preferences[2,3]. *In vivo,* however, nucleosome localization is subject to contributions from combinations of thermal motion, competitive protein binding and ATP-dependent remodelling, which cause sliding or temporary removal of the core histone octamer from the DNA[4,5]. Nucleosome mobility is further influenced by modifications to the histone octamer itself — such as exchange of histone variants or post-translational modifications. These can alter nucleosome properties or can serve to recruit chromatin-modifying proteins[6,7]. Replacement of canonical histones with variant forms may alter interaction surfaces and the overall stability of nucleosomes[8].

Recent advances in microarray and massively parallel sequencing technologies have enabled the generation of genome-wide profiles of nucleosome occupancy, DNA accessibility and histone modification patterns at an unprecedented coverage and accuracy[9–12]. These profiles have been generated using methods such as chromatin immunoprecipitation followed by microarray (ChIP–chip), chromatin immunoprecipitation followed by sequencing (ChIP–seq) and deoxyribonuclease (DNase) or micrococcal nuclease (MNase) digestion followed by sequencing (DNase–seq and MNase–seq). These genomic maps are revealing the prevalence of stereotypic nucleosome arrangements and modifications, which define distinct chromatin architectures at *cis*-regulatory sequences, including enhancers and promoters. Moreover, these studies have uncovered cell-type-specific chromatin signatures that suggest a dynamic interplay between tissue-specific regulation by transcription factors and chromatin structure. The extent to which these changes in DNA accessibility are a cause or a consequence of productive transcription-factor binding remains a central question.

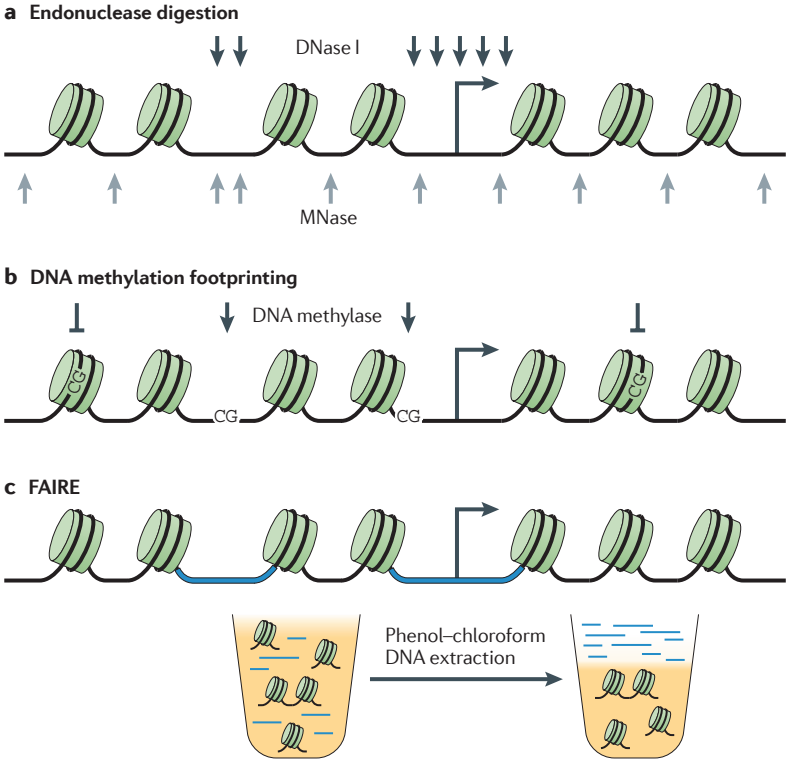
Box 1 | **Methods for studying accessibility**

DNase I digestion
This is a classical method to locate accessible chromatin structure. It capitalizes on the fact that DNA–protein interactions, such as nucleosome packaging, protect chromosomal DNA from cleavage by endonucleases (see part **a** of the figure; cleavage sites are shown by black arrows). Typically, this approach involves deoxyribonuclease I (DNase I) titration followed by characterization of the digested DNA by Southern blot analysis, although recent adaptations of the technique have used microarray and high-throughput sequencing technologies, thus enabling genome-scale mapping. Regions that have high sensitivity to digestion are called DNase I hypersensitive sites. This method has been particularly useful in identifying accessible *cis*-regulatory elements including promoters (indicated by the right-angled arrow in the figure) and enhancers of actively transcribed genes.

Micrococcal nuclease digestion
This is another endonuclease-based method, which generates cuts (see the figure, part **a**, grey arrows) preferentially within linker DNA between nucleosomes and in nucleosome-depleted regions. This method allows the determination of nucleosome occupancy and positioning. Coupled with microarrays and massively parallel sequencing, genome-wide micrococcal nuclease (MNase) digestion profiles have revealed that nucleosomes are not randomly distributed but can be highly positioned or depleted in certain genomic regions, in particular upstream and downstream of transcription units.

DNA methylation footprinting
In this method (see the figure, part **b**), bacterial DNA methyltransferases add methyl groups (black arrows) to sequence motifs (CG shown here) in exposed genomic sequences, but less efficiently methylate motifs that are occluded in the context of nucleosomes (indicated by the T bars). Methylation footprinting displays a broad sensitivity to chromatin structure ranging from detection of nucleosome-depleted loci to general accessibility of large chromosomal regions. Thus, this method complements chromatin analysis with conventional nuclease-based assays. Use of cytosine methylases followed by bisulphite treatment — which converts unmethylated cytosine to uracil — and sequencing can allow detection of the differential accessibility of individual DNA molecules.

Formaldehyde-assisted isolation of regulatory elements (FAIRE)
This method (see the figure, part **c**) entails formaldehyde fixation of chromatin and subsequent separation of protein-free DNA (blue segments) by phenol–chloroform extraction. The resulting soluble fraction is enriched for nucleosome-depleted DNA, including sites of DNase I hypersensitivity.



Here, we provide a broad overview relating different features of chromatin structure to genomic function and discuss the relevance of genome accessibility for the regulation of DNA-templated reactions. We introduce methods for studying chromatin structure and discuss what genome-wide approaches have revealed about global nucleosome patterns. We then consider important determinants of chromatin accessibility, including DNA sequence, ATP-dependent remodelling and nucleosome modifications. We focus more on nucleosome arrays and less on higher-order chromatin structures owing to the lack of understanding of the relationship between access and these different organizational levels of chromatin. Finally, we highlight the dynamic nature of chromatin by providing examples of how histone octamers are mobilized to change DNA accessibility in diverse cellular responses ranging from transcription to DNA replication and repair.

Defining and measuring accessibility

Our understanding of genome accessibility stems mostly from biochemical assays on isolated cell nuclei using enzymes that can digest or methylate exposed DNA. Differences in DNA access are revealed by the local catalytic activity of these enzymes. These useful, although indirect, methods have provided an operational definition of chromatin accessibility, which we use as a basis for discussion in this article. Methods that provide information about higher-order chromatin structure can give an indication of whether some regions of the genome are more or less condensed in three-dimensional space, but little is known about how higher-order structure relates to access of enzymes to the DNA template. Therefore, in this Review, we refer to condensation rather than accessibility when discussing higher-order chromatin structures.

Nuclease- and fragmentation-based methods. Nucleases, such as DNase I, are sensors of differential DNA access within chromatin. Sites of preferential digestion are referred to as DNase I hypersensitive sites and usually reside in *cis*-regulatory elements such as promoters and enhancers of actively transcribed genes[13–15] (BOX 1). These DNase I hypersensitive sites are characterized by higher turnover of histones and reduced nucleosome occupancy[16]. Nucleosome depletion also allows enrichment of *cis*-acting elements by the formaldehyde-assisted isolation of regulatory elements (FAIRE) assay. This technique fragments and isolates DNA that cannot be crosslinked to nucleosomes[17] (BOX 1). Both approaches have been adapted for genome-wide detection, and the results of genome-wide studies demonstrate that accessibility is a common property of most functional *cis*-regulatory elements in eukaryotic genomes[18,19].

In addition, nucleases have been used to determine the general sensitivity to digestion of chromatin outside hypersensitive sites. Although highly transcribed genes — such as the β-globin gene in chicken erythrocytes — show higher general sensitivity to nucleases than transcriptionally inactive genes or intergenic regions[20], the dynamic range of this assay is limited[21].

Nucleosome occupancy
The probability that a genomic site is covered by a histone octamer; this is an average frequency measure in a cell population.

Chromatin immunoprecipitation followed by microarray
(ChIP–chip). This is a technique that combines chromatin immunoprecipitation (ChIP) with detection on microarrays ('chip') to comprehensively investigate the distribution of a protein of interest. Protein–DNA complexes are immunoprecipitated and, after isolation, bound DNA sequences can be detected by hybridization to probes on a microarray chip.

Chromatin immunoprecipitation followed by sequencing
(ChIP–seq). An advancement of chromatin immunoprecipitation followed by microarray (ChIP–chip), ChIP–seq combines ChIP with massively parallel DNA sequencing to identify binding sites of a protein of interest genome-wide.

DNase I hypersensitive sites
Chromatin regions with frequent cleavage by DNase I. DNase I hypersensitivity generally reflects a local reduction in nucleosome occupancy.

Nucleosome positioning
This can describe either the rotational or translational orientation of the DNA around the histone octamer. Rotational positioning describes the orientation of the DNA helix on the surface of the histone octamer. Translational nucleosome positioning relates to the specific 146 bp of genomic DNA covered by the histone octamer. A highly positioned nucleosome is one that covers the same sequence in most cells within a population.

Bisulphite treatment
Treatment of DNA with bisulphite chemically converts unmethylated cytosines to uracil. As methylated cytosines are unaffected, the location of methylation can be identified by sequencing the bisulphite-treated DNA.

Nucleosome occupancy and nucleosome positioning can be measured using MNase digestion, which preferentially cleaves DNA in linker regions between nucleosomes to generate mono- and oligonucleosomes[22] (BOX 1). When combined with microarray or high-throughput sequencing, this approach has allowed accurate mapping of nucleosomes across genomes from yeast to human[11,12,23–25]. The resulting profiles indicate that nucleosomes tend to be characteristically positioned or depleted at regulatory regions in the genome, in particular at promoters and 3′ ends of transcription units.

Methylation footprinting approaches. Methylation footprinting with exogenous DNA methyltransferases provides an alternative approach to measure differential DNA accessibility[26–29] (BOX 1). Cytosine methylases such as *M.CviP*I or *M.Sss*I leave a methylation footprint at exposed sequences but are excluded by nucleosomes and other DNA-binding proteins. The resulting DNA methylation can be identified by bisulphite treatment and DNA sequencing, which, when combined with subcloning of individual DNA fragments, allows the methylation readout of single DNA molecules. Notably, the application of methylation footprinting is limited by the presence of endogenous DNA methylation in mammalian cells. In eukaryotes that lack endogenous methylation, this approach has nevertheless been successfully used to identify nucleosome positions and patterns of general accessibility[26,30].

Assaying higher-order chromatin structure. Accessibility studies with the above methods revealed hypersensitive and nucleosome-free regions as frequent features of functional regulatory regions. However, as noted above, these approaches have a limited dynamic range, which cannot discriminate chromatin structures associated with low DNA accessibility. For example, heterochromatic regions — which are actively repressed — are also predicted to be less accessible than the genomic average. Although studies using methylation footprinting found reduced methylase accessibility at domains marked with repressive histone modifications, the observed differences between these regions and those marked by active histone modifications are small[30]. Because current methods are mostly biased to measure changes in nucleosome arrays, it is largely unclear whether condensed heterochromatin domains are indeed associated with reduced DNA accessibility.

By contrast, chromatin compaction could involve higher-order organization. Such three-dimensional chromatin organization can be estimated by measuring the proximity of distal chromosomal sites. This has traditionally been done by hybridizing fluorescent probes to distal genomic sites (fluorescence *in situ* hybridization (FISH)) and measuring their proximity by microscopy. However, this technique is limited by its low resolution, which does not allow discrimination between close proximity and physical contact.

FISH studies of long-range interactions have recently been complemented by chromosome conformation capture (3C), a technique that involves crosslinking and physical ligation of DNA sequences that are juxtaposed in the context of chromatin[31,32]. The 3C technique measures physical interactions between distal chromatin regions at a higher resolution than conventional microscopy techniques. Combined with PCR, the initial 3C approach was limited to testing the proximity between a few selected sites only. More recently, 3C has been combined with detection by microarray and massively parallel sequencing, which has facilitated genome-wide identification of distal intrachromosomal or interchromosomal contacts[33–37]. Together, derivatives of 3C assays provide exciting insights into higher-order chromosome organization. However, it remains to be resolved whether proximity of individual loci reflects looping or general chromosomal condensation and how this relates to proteins gaining access to DNA.

Patterns of genome accessibility. Compared to higher-order structures, more is known about chromatin organization at the level of nucleosome arrays. DNase I hypersensitivity frequently coincides with reduced nucleosome occupancy over *cis*-regulatory regions[15,38]. Some sensitivity to nuclease digestion (but less than at regulatory regions) is also observed throughout actively transcribed genes[21,20]. This is likely to reflect transient DNA exposure as a consequence of transcription-dependent nucleosome turnover[39]. Inactive genes and intergenic chromosomal regions display only low susceptibility to DNase I digestion. The inability of nucleolytic methods to further resolve more condensed structures of chromatin thereby constitutes a major limitation. DNA methylase footprinting presents a complementary approach to DNase I digestion by offering a different range of chromatin sensitivity. Indeed, genomic methylase footprinting in *Drosophila melanogaster* revealed the greatest DNA accessibility at genomic regions of histone H4 lysine 16 (H4K16) hyperacetylation (a modification associated with active genes), whereas transcriptionally repressed domains demarcated with H3K27 methylation were the least accessible to methylase activity[30]. Nevertheless, there is an apparent lack of methodologies to address the detection gap between nuclease insensitivity and the highly condensed subnuclear structures of heterochromatin that warrants the development of novel approaches.

Molecular basis of nucleosome positioning

Because the nucleosome presents the primary determinant of DNA accessibility[40,41], it is crucial to understand the rules underlying nucleosome positioning *in vivo*. By definition, nucleosome positioning refers to the localization of an individual histone octamer with respect to a specific DNA sequence; conversely, nucleosome occupancy denotes an average frequency measure in a cell population. First, we discuss the influence of the DNA sequence itself, and then we introduce a range of factors that modulate accessibility. Throughout the discussion, we provide examples highlighting the roles of various activities in regulating nucleosome location and mobility.

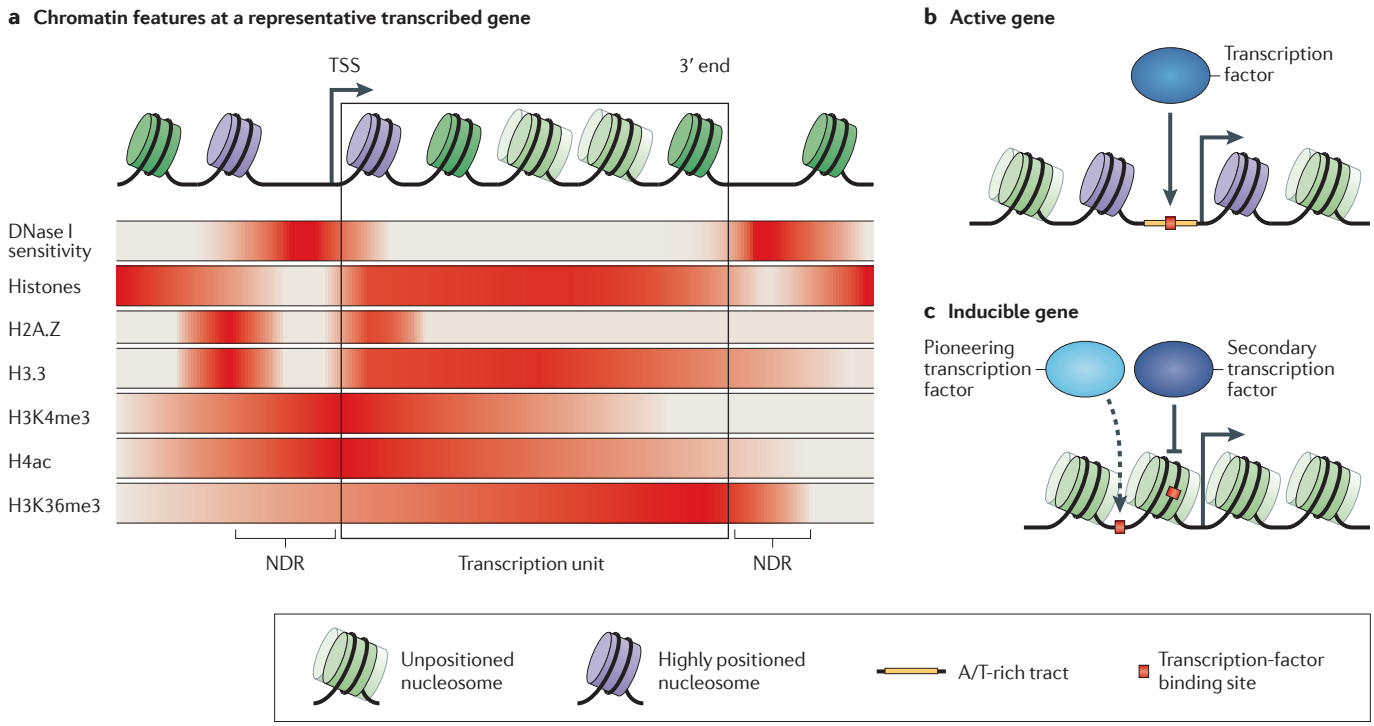


Figure 1 | **Chromatin structure and DNA accessibility at genes. a** | Schematic view of primary chromatin features at a hypothetical transcribed gene. Heat maps (high = red, low = beige) indicate the density and location of the chromatin features listed on the left. Active genes are typically characterized by regions of low nucleosome occupancy (nucleosome depleted regions (NDRs)), which coincide with DNase I hypersensitive sites and are located upstream and downstream of the transcription unit (boxed area). Flanking the NDR at the promoter (which is indicated by the right-angled arrow, labelled TSS for 'transcription start site') are two highly positioned nucleosomes, which exhibit histone H4 acetylation (H4ac) and H3 trimethylation at lysine 4 (H3K4me3) and frequently contain histone variants H3.3 and H2A.Z. H3.3 is also deposited downstream in the gene body. H3K36me3 accumulates in the gene body towards the 3′ end. **b** | Nucleosome occupancy at a hypothetical constitutively active promoter. An A/T-rich tract disfavours stable nucleosome formation and enhances exposure of the binding site to facilitate transcription-factor interaction within the NDR. **c** | Nucleosome occupancy at a hypothetical repressed promoter. Transcription-factor binding sites are typically embedded within nucleosomes that occlude interaction. Nucleosome mobilization is required for transcriptional activation and involves initial binding of the pioneering transcription factor within the nucleosome linker region, which, in turn, recruits histone modifiers and ATP-dependent chromatin remodellers to expose additional binding sites for a secondary transcription factor.

Fluorescence *in situ* hybridization
(FISH). A technique that can be used to visualize the location of DNA sequences within the nucleus by using sequence-specific fluorescent probes and microscopy.

Chromosome conformation capture
(3C). A technique used to study the spatial organization of chromosomal regions *in vivo*, based on the ligation of DNA elements that are in close physical proximity.

Nucleosome-depleted regions
(NDRs). Sites of reduced nucleosome occupancy compared to immediate surrounding regions. NDRs are frequently located at the beginning and end of genes, harbour *cis*-regulatory binding sites and display sensitivity to DNase I and formaldehyde-assisted isolation of regulatory elements (FAIRE) detection.

Sequence determinants of nucleosome occupancy. Genome-wide studies have revealed that nucleosomes can be locally depleted or highly positioned, meaning that not only position but also occupancy can be site specific[11,12,24]. Most transcribed genes have reduced nucleosome occupancy over the promoter, and these nucleosome-depleted regions (NDRs) are generally flanked by two well-positioned nucleosomes (FIG. 1a). Evidence for pro- and antinucleosomal DNA sequences has been previously reported, but the extent to which genomic DNA can determine chromatin structure *in vivo* is unclear[42–44]. As the DNA has to bend sharply around the surface of the histone octamer, nucleosome formation is favoured by flexible or intrinsically curved sequences, whereas more rigid, less flexible sequences are unfavourable for histone-octamer incorporation. Indeed, poly(dA) stretches, which are intrinsically stiff, have been shown to disfavour nucleosome formation *in vitro*[2,3]. Moreover, in some lower organisms, including budding yeast and worms, these 'antinucleosomal' sequences are overrepresented at sites of nucleosome depletion *in vivo*[2,45]. By contrast, histone-octamer exclusion by poly(dA) sequences has a substantially less important role in nucleosome positioning along fly and human chromosomes[24,25]. DNA analysis of well-positioned nucleosomes has revealed a statistically significant enrichment of A/T dinucleotides in 10 bp intervals[9,11,24]. Periodic A/T dinucleotide spacing has been suggested to bend the DNA, creating a consistent curvature that gives rise to an intrinsically stable nucleosome. Such nucleosome-positioning sequences appear to contribute to the rotational setting of the DNA helix on the surface of the histone octamer.

How do these sequence preferences influence the precise nucleosome localization along the genome *in vivo*? This question was addressed by comparison of *in vivo* data sets from budding yeast with the genome-wide distributions of nucleosomes reconstituted *in vitro*[2,3]. Although two initial studies reported that *in vitro* reconstitution captures aspects of

nucleosome organization — particularly the organization of nucleosomes upstream and downstream of genes — they came to different conclusions about the actual degree of positioning information that is encoded in the genome. Direct support for the idea that sequence features determine only a minor part of the actual nucleosome localization observed *in vivo* was provided in a recent study showing that *in vitro* reconstitution of nucleosome positioning outside yeast promoters required ATP-dependent *trans*-acting factors[46]. Together these results highlight our limited ability to predict nucleosome positioning from DNA sequence alone, but they do suggest that *trans*-acting proteins — which are currently not included in prediction models — have a major role in determining the precise nucleosome positioning and occupancy *in vivo*.

Modulators of genome accessibility. Nucleosome patterns that are upstream of transcription start sites most likely reflect the interplay between sequence determinants and chromatin modifiers. Post-translational histone modifications and ATP-dependent remodelling have emerged as important modulators of chromatin structure and nucleosome dynamics[47]. Their roles in transcription initiation and elongation are discussed in the following sections. Unlike canonical accessibility for the transcriptional machinery upstream and downstream of genes, DNA repair proteins need to gain access to the genome at any site at which a lesion occurs. Moreover, initiation of DNA replication relies on binding of the machinery to suitable start sites and subsequent processing throughout the genome. We focus mainly on transcription, as the impact of accessibility has been more extensively studied in this context.

Activation of transcription

Transcription factors. Sequence-specific binding of transcription factors is the key specifying event for gene activation. In *Saccharomyces cerevisiae*, transcription-factor binding sites are typically found within NDRs located upstream of transcribed genes and are thus readily accessible[11] (FIG. 1b). Promoters of repressed genes, however, are frequently embedded in nucleosomes (FIG. 1c). We still have no comprehensive understanding of how transcription factors cope with chromatin, yet interesting examples highlight the potential differences in their sensitivity and interaction with DNA in the context of nucleosomes. For instance, transcription-factor binding to nucleosomal DNA can lead directly to displacement of histone octamers *in vitro*[48]. However, most transcription factors require exposure of their binding sites either within linkers or by nucleosome mobilization[49,50]. The glucocorticoid receptor, a ligand-activated transcription factor, binds largely to pre-existing DNase I hypersensitive sites. These sites mostly rely on ATP-dependent nucleosome remodelling activity and are cell-type-specific, providing a potential mechanism for how accessibility of chromatin might facilitate tissue-specific targeting of the glucocorticoid receptor[51].

Alternatively, transcription-factor binding sites covered by nucleosomes can become exposed during spontaneous unwrapping and rebinding of the histone octamer[52,53]. Frequently, repressed promoters harbour at least one accessible binding site to facilitate interactions with 'pioneering' transcription factors before induction of local chromatin changes. A well-studied example is the *PHO5* promoter in yeast, which contains one exposed Pho4-binding site located in the linker between two nucleosomes, whereas additional binding sites are buried within nucleosomes[54]. During induction, Pho4 binds to the accessible site first and recruits proteins with histone-modifying and nucleosome-remodelling activities, which, in turn, expose the secondary binding sites (FIG. 1c). The binding affinity of the primary Pho4 sites is particularly important for sensing cellular phosphate levels and inducing subsequent chromatin changes to allow transcription initiation[55]. These important findings have led to a model based on the interplay between the affinity and accessibility of transcription-factor binding sites to explain how various genes of the phosphate-response pathway differentially respond to cellular levels of inorganic phosphate[55]. The intricate relationship between nucleosome organization and fine-tuning of transcription thereby highlights the relevance of nucleosome mobilization as a rate-limiting step in transcription initiation.

ATP-dependent nucleosome remodelling complexes. Various enzymatic machines can perturb intrinsic histone–DNA interactions. These nucleosome remodellers are multiprotein complexes that use ATP hydrolysis to slide or disassemble histone octamers[56,57] (FIG. 2a). A well-studied example is the nucleosome remodelling complex RSC, which is required for the transcriptional activation of most yeast genes[58]. At target promoters, RSC nucleosome remodelling activity might explain the discrepancy between sequence-based prediction and the actual nucleosome localization. In the absence of RSC, NDRs shrink and flanking nucleosomes become poorly positioned, which suggests that RSC can synergize with DNA-intrinsic properties[59]. Indeed, interaction between the Myb-family transcription factor Reb1 and its binding site is facilitated by the presence of a poly(dT) tract, and this process precedes RSC recruitment and nucleosome remodelling[60]. This supports a stepwise mechanism with transcription-factor binding being the primary event, followed by recruitment of a remodelling complex to mediate proper NDR formation and positioning of flanking nucleosomes. By contrast, the chromatin remodelling complex Isw2 in yeast antagonizes NDR formation by sliding nucleosomes onto unfavourable A/T-rich DNA tracts. By overriding nucleosomal sequence preferences, Isw2 serves to suppress unwanted transcription that could otherwise arise from nucleosome-depleted cryptic start sites[61].

The emerging picture suggests that DNA sequence properties can facilitate formation of nucleosome-depleted promoters, although the actual position of nucleosomes is mostly influenced by the activity of *trans*-acting proteins, such as transcription factors and ATP-dependent remodellers, which interact with the chromatin template.

RSC
A multi-subunit chromatin remodelling complex that uses DNA-dependent ATP hydrolysis to catalyse nucleosome mobilization at active genes.

Chromatin remodelling
Enzyme-assisted histone or nucleosome mobilization, which requires ATP hydrolysis. ATP-dependent chromatin remodelling influences local chromatin structure to facilitate or prevent protein accessibility, which is required to initiate DNA-templated reactions.

Overcoming the chromatin barrier during transcription elongation. The presence of nucleosomes not only interferes with transcriptional activation but also reduces template accessibility for elongating RNA polymerase (Pol) II. Pol II is inefficient at elongating through a nucleosomal template *in vitro*, so nucleosomes located over the gene body have to be mobilized to allow transcription[62]. Indeed, several studies provide evidence that nucleosomes can be lost during transcription. Genome-wide mapping of nucleosomes in yeast showed reduced nucleosome occupancy over promoters but also partial loss of nucleosomes at the coding regions of highly transcribed genes[63]. Polymerase-dependent histone displacement requires the activity of several histone chaperones. For example, *in vitro* studies suggest roles for FACT and the histone chaperone Asf1 in core-histone displacement[64,65]. In turn, the transcription elongation factor Spt6 seems to be required for the assembly of evicted histones to re-establish normal chromatin structure[66]. Interestingly, lack of all three histone chaperones results in aberrant transcription from cryptic start sites within transcribed coding regions[65,67,68]. Thus, these chaperones function as histone donors and enable chromatin reconstitution after polymerase passage.

The influence of histone variants. Changes in histone-octamer composition by incorporation of histone variants add to the complexity of chromatin and can also affect DNA accessibility. Histone variants are separately encoded and differ in sequence to canonical histones. For example, histone H3.3 is almost identical to canonical H3 with only four amino acid changes, whereas the centromere-specific variant CENP-A only shares similarity within the histone-fold domain[8]. Importantly, even small amino acid differences can have profound effects on histone properties. In contrast to canonical histones, which are synthesized only during the S phase, H3.3 variants are synthesized outside the S phase of the cell cycle, become incorporated into nucleosomes and are deposited at specific locations primarily in a replication-independent manner[8] (FIG. 2b). The histone variant H3.3 is highly enriched for several modifications associated with transcription and is specifically incorporated at transcribed genes and regulatory sequences[16,69–71]. This deposition presumably compensates for nucleosome displacement caused by protein binding or polymerase passage[70,71].

In a similar manner to H3.3, H2A.Z is specifically assembled into promoter nucleosomes, replacing canonical H2A in a replication-independent manner. The role of H2A.Z in transcriptional regulation is still debated, as the precise location and timing of placement varies in different organisms[9,24,60,72]. However, the commonly observed deposition of H2A.Z within NDR-flanking nucleosomes suggests a role in either establishing or maintaining a permissive chromatin structure at gene promoters. In agreement with this model, octamers containing both histone variants (H2A.Z and H3.3) form less stable nucleosomes, which is likely to facilitate eviction and promotes accessibility for subsequent rounds of transcription initiation[73]. Thus, deposition of histone variants not only appears to compensate for nucleosome loss but might also destabilize nucleosomes to maintain accessible chromatin.

Chemical modifications of histones. Histones are subject to a wide range of reversible post-translational modifications. Depending on the modification and targeted residue, these can function as docking sites for *trans*-acting factors or influence the structural organization of chromatin and thus affect DNA accessibility. Modifications associated with the active state, such as H3K4 trimethylation (H3K4me3) and H4 acetylation (H4ac), localize in a stereotypic pattern to promoters and 5′ regions of most transcribed genes, where they overlap with DNase I hypersensitive sites and H3.3-containing nucleosomes[47,74] (FIG. 1a). Promoter localization of these marks can create docking sites for recruitment of chromatin-modifying proteins. Recent studies have identified numerous protein domains that are able to specifically

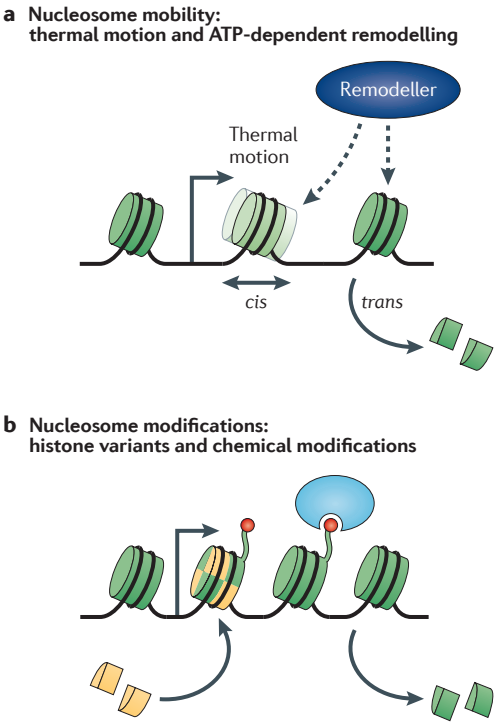


Figure 2 | **Mobility and stability of nucleosomes.** **a** | Nucleosome mobility is influenced by thermal motion and the activity of ATP-dependent nucleosome remodelling complexes. Spontaneous unwrapping can displace nucleosomes in *cis* (that is, along the DNA). ATP-dependent chromatin remodellers can slide in *cis* or displace the histone octamer (that is, in *trans*). **b** | Nucleosome stability is affected by the octamer composition and modification pattern of histones. Incorporation of histone variants (yellow) may alter interactions with histone and non-histone proteins. Post-translational histone modifications (red circles) can lead to the recruitment of chromatin-modifying proteins (blue oval) and *in vitro* evidence suggests that they can alter electrostatic interactions between histones and DNA and between neighbouring nucleosomes.

FACT
Stands for 'facilitates chromatin transcription' and is a chromatin-specific histone chaperone that is required for transcriptional elongation through chromatin templates.

bind histone tails that are methylated (chromodomains, PHD domains, Tudor domains and WD40 domains) or acetylated (bromodomains)[75]. For example, the PHD domain of the nucleosome-remodelling factor subunit BPTF can specifically recognize H3K4me3. Being the largest subunit of the nucleosome-remodelling complex NURF, this BPTF interaction recruits nucleosome-remodelling activity to disrupt chromatin at the promoter and enhance transcription initiation[76] (FIG. 2b).

Alternatively, histone modifications are thought to facilitate nucleosome eviction by affecting the nucleosome net charge and by reducing electrostatic interactions between histones and DNA or between histones of neighbouring nucleosomes[77,78]. This is exemplified by H4K16ac, which has direct effects on DNA accessibility but is also recognized by *trans*-acting factors. *In vitro*, H4K16ac disrupts electrostatic interactions between the amino-terminal tail of H4 and an acidic patch of the H2A/H2B dimer of an adjacent nucleosome, thus preventing the formation of compact 30 nm fibres[77,78]. In addition, the nucleosome-remodelling complex ACF engages the H4 tail but is repelled by acetylation at lysine 16, indicating an additional role of this modification that is independent of its effects on electrostatic interactions[78]. In *Drosophila melanogaster*, H4K16ac is highly enriched on the male X chromosome and is crucial for its transcriptional upregulation as part of dosage compensation[79]. The electrostatic effects and exclusion of repressive nucleosome-remodelling activity observed *in vitro* might contribute to the increased transcriptional output. Indeed, the presence of H4K16 hyperacetylation correlates well with greater DNA accessibility at gene bodies and increased escape of RNA Pol II from the promoter, resulting in productive elongation[30,80]. Recently, direct effects on chromatin compaction have also been reported *in vitro* for H2B ubiquitylation (H2Bub)[81]. However, unlike acetylation, ubiquitin is a bulky peptide that might pose a steric interference, which prevents nucleosome stacking and condensation into regular 30 nm fibres.

Transcriptional repression

Some post-translational histone modifications and *trans*-acting protein modifiers have also been implicated in the silencing of gene expression. In analogy to increasing accessibility for gene activation, repressive chromatin modifications are thought to induce formation of condensed chromatin, which restricts transcription. However, many questions remain unanswered, which is mostly due to the challenges of assaying DNA accessibility that are associated with compact chromatin structures in living cells.

Heterochromatin. Genomic regions of constitutive heterochromatin typically remain condensed throughout the cell cycle[82] and are characterized by histone hypoacetylation and H3K9me and H4K20me[83,84]. Outside constitutive heterochromatin, H3K9me is also enriched and required for transcriptional silencing of genes and retroviral repeat elements[85–87]. Chromatin compaction and gene repression involves recruitment of heterochromatin protein 1 (HP1). HP1 specifically recognizes and binds methylated H3K9 and can oligomerize to bridge nearby nucleosomes. Thus, H3K9me-dependent repression is thought to involve nucleosome scaffolding by HP1, and there is evidence that this can generate a condensed chromatin template, thereby raising the possibility that it could reduce protein accessibility to the genomic template[88–90]. H4K20me3, however, was recently shown to induce chromatin condensation *in vitro* by itself[91]. This could point to an intrinsic ability of H4K20me3 in enhancing compaction, which potentially synergizes with the H3K9me–HP1 system and further facilitates condensation of heterochromatic fibres *in vivo*.

Polycomb-mediated gene repression. The Polycomb group proteins form a class of factors that was originally described in *D. melanogaster* as being responsible for maintaining stable and heritable repression of patterning genes, including homeotic genes. Since then, Polycomb action has been found to repress key developmental regulators from insects to mammals[92]. Although it is known that Polycomb function results in modification of histones, the molecular mechanism of silencing remains unclear. H3K27me3 is catalysed by a subunit of Polycomb repressive complex 2 (PRC2)[93,94] and PRC1 mediates H2A119ub. It has been proposed that H3K27me3 signals for PRC1 recruitment at target promoters and that subsequent ubiquitylation represses transcription by interfering with polymerase elongation[95]. PRC1 components can promote compaction of nucleosomal arrays *in vitro*[96] and can mediate long-range interactions *in vivo*, supporting a role for Polycomb proteins in establishing repressive higher-order chromatin structure[36,97]. Interestingly, a recent report[98] suggests that PRC1 complexes that lack ubiquitylation activity are still able to silence target genes and mediate chromatin compaction. Therefore, an alternative scenario might entail Polycomb proteins establishing a physical barrier that interferes with binding of the transcriptional machinery. Indeed, experiments using DNA methylase footprinting in *D. melanogaster* revealed reduced DNA accessibility at H3K27me3 domains, which is in agreement with the notion that Polycomb-dependent repression reflects a change in chromatin compaction and organization[30]. Additional experiments will be needed to discern whether Polycomb repression reflects localized hindrance of polymerase elongation, chromatin condensation or possibly a combination of both modes of regulation.

In extension of this point, the development of novel experimental approaches appears to be necessary to measure chromatin compaction in heterochromatin. For instance, using 3C in conjunction with ChIP of repressive modifiers and modifications might link condensation of distal genomic sites to the localization of underlying repressive chromatin modifications[34,36]. Similarly, further efforts to map regulatory and structural proteins that are involved in mediating chromatin compaction, such as lamins and cohesins, would complement existing localization data and could potentially bridge the gap between primary nucleosome arrays and nuclear organization[99,100].

PHD domains
Derived from the name 'plant homeodomain', these protein domains were initially discovered as a Cys_4-His-Cys_3 motif in the homeodomain protein HAT3 in *Arabidopsis thaliana*. They are present in many proteins, several of which are nuclear and involved in chromatin-mediated gene regulation.

30 nm fibres
An array of nucleosomes (often called 'beads on a string') wraps into a more condensed fibre, which has a diameter of 30 nm. A simple 30 nm fibre has been reconstituted *in vitro*, but its actual composition *in vivo* remains unclear.

Constitutive heterochromatin
Genomic regions, predominantly at centromeres and telomeres, which remain condensed throughout the cell cycle. These often consist of highly condensed, repetitive DNA and are largely transcriptionally silent.

Polycomb group proteins
An evolutionarily conserved set of proteins that regulate the temporal and spatial expression pattern of key developmental genes through modulation of chromatin structure.

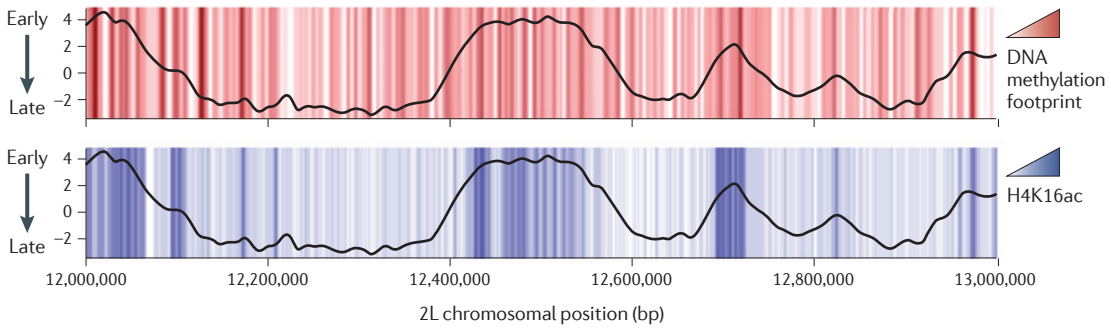


Figure 3 | **Local chromatin structure relates to DNA replication timing.** These chromosomal profiles show DNA replication timing (black line) in *Drosophila melanogaster* tissue culture cells. The underlying heat maps reveal that early replication in the S phase correlates with hyperacetylation of histone 4 at lysine 16 (H4K16ac, bottom panel) and increase DNA accessibility (top panel) as shown by methylase footprinting. White indicates the absence of enrichment; darker colours indicate higher levels. This figure is modified, with permission, from REF. 30 © (2010) Macmillan Publishers Ltd. All rights reserved.

Beyond transcription

So far, we have focused on the well-established impact of chromatin organization on transcriptional regulation, although at least two other fundamental processes require access to DNA in the context of chromatin. They are the initiation of DNA replication and the repair of DNA lesions.

Accessing the genome for DNA replication. Replication of eukaryotic genomes occurs in a temporally ordered fashion that is, in part, cell-type specific[101]. This program is a consequence of the location of origins of replication (ORIs) — the sites at which replication is initiated — and their time of firing during the S phase. In yeast, ORIs are largely defined by a DNA sequence motif that is recognized by the origin-recognition complex (ORC). Even though this complex is highly conserved, no ORI-consensus DNA motif has yet been determined in higher eukaryotes[102]. If such a motif exists, it might be complex, and its identification would require extremely accurate genome-wide maps of initiation sites. Alternatively, replication origins in higher eukaryotes might be primarily defined by chromatin structure[103]. Indeed, in *D. melanogaster,* ORCs occupy sites that are characterized by nucleosome depletion and high turnover of H3.3, thus showing links between chromatin marks and DNA replication[104]. Similarly, limited analysis of replication initiation in the human genome has identified locations of ORIs in proximity or overlapping with CpG-rich promoter elements. As many of these promoters are found to be actively transcribed[105] and are thus accessible, this evidence lends further support to the idea of a chromatin-mediated connection between transcriptional and replicative regulation.

Using yeast DNA with purified ORCs and a remodelling complex, chromatin-reconstitution experiments have reproduced the positioning of nucleosomes observed around ORCs *in vivo*[106]. However, these *in vitro* experiments have not yet provided the order of events. In *D. melanogaster*, genomic profiles of histone modifications and chromatin components are predictive of the location and activity of ORIs[104]. Further correlative evidence comes from studies in which maps of replication timing were contrasted with chromatin modifications (FIG. 3). For example, in flies, the transcriptionally upregulated male X chromosome is uniquely marked by high levels of H4K16ac, which coincide with a shift towards earlier replication of the entire chromosome, compared with the female X chromosome[107]. Dramatic shifts in replication timing have also been observed during stem cell differentiation and were suggested to reflect differential chromatin and nuclear organization[108].

These recent data sets argue that the replication program responds to local changes in chromatin structure, and that chromatin is thus involved in specifying sites of replication initiation in higher eukaryotes. Understanding the exact regulation of ORIs will require detailed genomic maps of their location. Based on the current evidence, it can be speculated that ORI regulation by chromatin structure will be less strict than regulation of initiation of transcription. Active ORIs tend to cluster in the genomes of higher eukaryotes[109], and the absence of activity of one is likely to be compensated by the activity of the neighbouring ORI. Such redundancy might potentially buffer the system and contribute to efficient replication of the very large eukaryotic genomes.

Gaining accessibility during DNA damage repair. DNA damage can occur throughout the genome and, in contrast to initiation of transcription, DNA repair requires access to DNA regardless of sequence and chromatin state. Our current understanding of this process is perhaps best demonstrated by the nucleotide excision repair (NER) pathway.

The global genome repair branch of NER detects and repairs UV-light-induced photodimers and bulky DNA base adducts. This repair pathway has been studied in much detail and, importantly, has been fully reconstituted *in vitro*[110]. These studies established that the core NER machinery only functions in the absence of chromatin; the presence of nucleosomes impairs multiple stages of the repair process, including damage detection,

Origin-recognition complex
A multi-subunit protein complex that binds to origins of replication and that is essential for initiation of replication.

Nucleotide excision repair
A versatile repair pathway that is involved in the removal of the most bulky DNA lesions, such as UV-induced thymine dimers and 6-4 photoproducts. If left unrepaired, these lesions stall transcription and can only be repaired through potentially error-prone translesion polymerases. Mutations in this pathway result in premature ageing syndromes, as well as cancer predisposition.

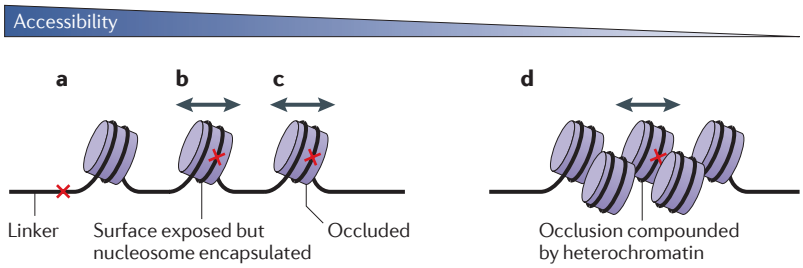


Figure 4 | **Creating access for DNA repair.** DNA lesions (red crosses) require repair irrespective of their position in the genome. Four different scenarios are depicted. **a** | Lesion located in a nucleosomal linker and access is only restricted by neighbouring nucleosomes. **b** | Damage is located within the nucleosome, but the surface is exposed. **c** | Lesion is embedded in the nucleosome pointing towards the octamer core, so it is not accessible on the surface. **d** | The situation is the same as in **c**, but the lesion is embedded in heterochromatin. The 'breathing' motion of nucleosomes and positional movement resulting from chromatin-remodeller background activity are indicated by the horizontal arrows.

DNA excision and DNA resynthesis[111,112]. Repair requires a fully assembled repair complex that has a footprint of approximately 100 bp on linear DNA, which is longer than the typical linker region between nucleosomes[113]. Based on these findings, it has been suggested that the nucleosome architecture inhibits functional NER complex assembly[114]. Cells in which chromatin remodelling complexes have been deleted become UV sensitive[115], which indicates that nucleosome remodellers facilitate NER in the context of chromatin. Reported interactions between NER damage-detection factors and chromatin remodellers[116–119] offer a potential means for recruitment of repair complexes despite their obstruction by chromatin. This mechanism nevertheless requires that DNA damage is detected in a manner that is compatible with chromatin. One of the main damage sensors in global genome NER is the XPC complex (which contains XPC and RAD23), but this complex is unable to bind to photodimers that are embedded in nucleosomes[120]. Other detection factors that are specifically tailored to chromatin must therefore exist. One candidate is the UV-damaged-DNA binding (UV-DDB) complex, a damage-detection sentinel that localizes to mononucleosomes following UV irradiation[121]. Based on structural analysis, a model has been proposed in which this complex can recognize its substrate on a nucleosome[122,123]. This model is likely to require a certain amount of flexibility in the nucleosomal organization, either by thermal motion or nonspecific chromatin remodelling (FIG. 4). UV-DDB2 is tightly linked to the cullin 4 (CUL4) E3 ubiquitin transferase, which ubiquitylates (among other targets) histones that surround the site of damage[124]. The exact role of ubiquitylation is not yet understood, but addition of this 'bulky' modification might interfere with nucleosomal compaction.

Spontaneous and enzyme-catalysed nucleosomal dynamics are expected to occur at lower rates in heterochromatin, in which repair does indeed proceed at a slower rate. Repair thereby suggests a possible paradigm for DNA access, as the DNA-recognition event must be performed in a largely chromatin-insensitive manner and with subsequent local changes in accessibility that facilitate the arrival of chromatin-sensitive components.

Conclusions

Genomic studies in different cell types that have combined nuclease digestion and ChIP with microarray or high-throughput sequencing have provided steady-state maps of nucleosome localization, composition and histone modifications. These have established general rules but have also identified differences between cell types. Kinetic studies at individual loci, such as the above discussed regulation of the *PHO5* promoter, further showed a functional role for nucleosome organization in regulating genome accessibility[55]. The emerging picture is that local nucleosome positioning and occupancy is largely determined by the combined action of DNA sequence features, transcription factors, nucleosome remodellers and histone modifiers. The resulting nucleosome arrangement directly affects sequence accessibility for protein–DNA interactions. Although the current evidence establishes functional relevance for chromatin changes, we still lack most of the molecular connections in this complex network. In particular, the order of events that define chromatin states and gene activity remains poorly understood.

Several studies support the notion that binding of a 'pioneering' protein to DNA constitutes the primary event in DNA-templated reactions (such as transcription and repair), which, in turn, leads to chromatin changes and exposure of additional regulatory sites. This model implies that pioneering factors exist that can interact with DNA sequences, even when they are wrapped in nucleosomes. Access for these factors might be facilitated by DNA sequence determinants that reduce nucleosomal occupancy or, alternatively, might originate from an opportunistic interaction during spontaneous nucleosome unwrapping. To determine what regulatory principles are used throughout the genome will require the experimental measurement of sequence preference and chromatin sensitivity for more DNA-binding factors. Together with genome-wide chromatin maps, such studies should contribute to predictive models of genome regulation that include chromatin accessibility.

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Competing interests statement
The authors declare no competing financial interests.

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