EPIGENETICS

Chromatin accessibility and the regulatory epigenome

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Abstract | Physical access to DNA is a highly dynamic property of chromatin that plays an essential role in establishing and maintaining cellular identity. The organization of accessible chromatin across the genome reflects a network of permissible physical interactions through which enhancers, promoters, insulators and chromatin-binding factors cooperatively regulate gene expression. This landscape of accessibility changes dynamically in response to both external stimuli and developmental cues, and emerging evidence suggests that homeostatic maintenance of accessibility is itself dynamically regulated through a competitive interplay between chromatin-binding factors and nucleosomes. In this Review, we examine how the accessible genome is measured and explore the role of transcription factors in initiating accessibility remodelling; our goal is to illustrate how chromatin accessibility defines regulatory elements within the genome and how these epigenetic features are dynamically established to control gene expression.

Chromatin-binding factors
Non-histone macromolecules that bind either directly or indirectly to DNA.

Transcription factor
(TF). A non-histone protein that directly binds to DNA.

Architectural proteins
Proteins that have a structural role in organizing chromatin, including linker and core histone proteins, as well as insulator proteins.

Chromatin accessibility is the degree to which nuclear macromolecules are able to physically contact chromatinized DNA and is determined by the occupancy and topological organization of nucleosomes as well as other chromatin-binding factors that occlude access to DNA. The nucleosome — a core structural element of chromatin — consists of an octamer of histone proteins encircled by ~147 bp of DNA[1–8] (see Olins and Olins[9] for an excellent historical review). The composition and post-translational modification of nucleosomes reflect distinct functional states[10] and regulate chromatin accessibility through a variety of mechanisms, such as altering transcription factor (TF) binding through steric hindrance[10] and modulating nucleosome affinity for active chromatin remodellers[11]. The topological organization of nucleosomes across the genome is non-uniform: while densely arranged within facultative and constitutive heterochromatin, histones are depleted at regulatory loci, including within enhancers, insulators and transcribed gene bodies[12,13]. Internucleosomal DNA is often bound by TFs, RNA polymerases or architectural proteins with linker histones, which facilitate higher-order chromatin organization[14–21] and regulate access to DNA. Nucleosome occupancy and linker histone occupancy are variably dynamic[22] across the genome, creating an accessibility continuum that ranges from closed chromatin to highly dynamic, accessible or permissive chromatin (FIG. 1). This landscape of chromatin accessibility broadly reflects regulatory capacity — rather than a static biophysical state — and is a critical determinant of chromatin organization and function.

The accessible genome comprises ~2–3% of total DNA sequence yet captures more than 90% of regions bound by TFs (the Encyclopedia of DNA elements (ENCODE) project surveyed TFs for Tier 1 ENCODE lines)[13]. With the exception of a few TFs that are enriched within either facultative or constitutive heterochromatin, the overwhelming majority of TFs surveyed within the ENCODE project bind to open chromatin almost exclusively[13]. TFs dynamically compete with histones and other chromatin-binding proteins to modulate nucleosome occupancy and promote local access to DNA[13,23,24]; in turn, the accessibility landscape of a cell type modulates TF binding[25–28]. For multicellular systems, TFs have a broad range of functional roles, providing dynamic regulation of transcription on short timescales and establishing and maintaining persistent epigenetic canalization of cell types that share a common genome. Consequently, chromatin accessibility reflects both aggregate TF binding and the regulatory potential of a genetic locus. This perspective establishes an analytical foundation for tracing changes in accessibility to differential binding of transcriptional regulators that determine cellular state.

Recent technological advances have dramatically broadened the application space of chromatin accessibility measurements by reducing biological material requirements to levels available clinically and by improving the discriminative capacity of these assays to identify putative regulatory domains at both single-cell and single-molecule resolution[13,23,29–35]. Our principal aims in this Review are to provide an overview of recent

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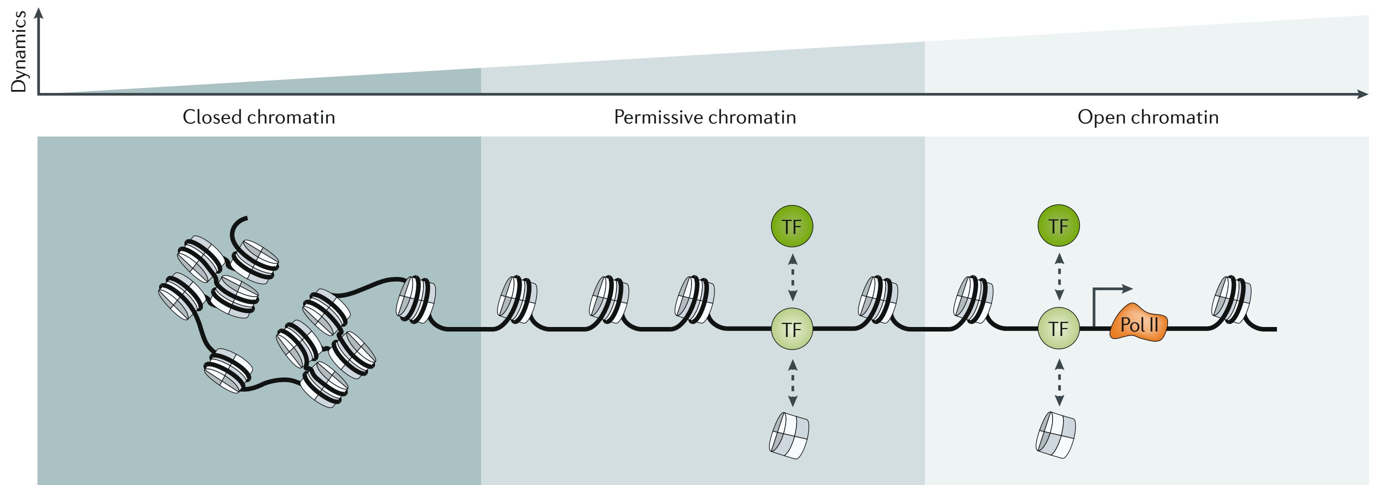

Fig. 1 | **A continuum of accessibility states broadly reflects the distribution of chromatin dynamics across the genome.** In contrast to closed chromatin, permissive chromatin is sufficiently dynamic for transcription factors to initiate sequence-specific accessibility remodelling and establish an open chromatin conformation (illustrated here for an active gene locus). Pol II, RNA polymerase II; TF, transcription factor.

advances in population-scale and single-cell measurements of chromatin accessibility, describe the principal biophysical determinants of accessibility and discuss the role of TFs in regulating accessibility at the nucleosome length scale. We conclude by briefly discussing the functional consequences of chromatin accessibility and potential directions for future research.

Measuring chromatin accessibility

Chromatin accessibility is almost universally measured by quantifying the susceptibility of chromatin to either enzymatic methylation or cleavage of its constituent DNA (FIG. 2). In principle, measurements of chromatin accessibility are dependent on the molecule for which access is being interrogated; however, remarkable conservation of accessibility has been reported across a diverse range of molecular probes[29]. In 1973, Hewish and colleagues used DNA endonucleases to fragment chromatin, showing that nucleosomes confer periodic hypersensitivity across the genome[36]. This periodicity was probed with Southern blot hybridization, showing a canonical 100–200 bp phasing pattern among DNase hypersensitivity sites (DHSs) that is conserved across genomic loci. This and subsequent work[37,38] provided the earliest direct evidence for stereotypical nucleosome phasing. Similar techniques were used to link chromatin remodelling with contemporaneous transcriptional activation of the heat shock locus in *Drosophila melanogaster*[39]. Following the introduction of PCR in 1985 (REF.[40]), a variety of quantitative methods (described below) have been developed to measure site-specific chromatin accessibility using endonucleases and ligation-mediated PCR[41,42].

DNase-seq. Genome-scale measurements of open chromatin were first reported in 2006 in a pair of studies that hybridized DNase I-cleaved fragments isolated from native chromatin onto tiled microarrays spanning 1% of the human genome[43,44]. Similar workflows were then adapted to quantify the relative abundance of DNase-sensitive chromatin across the genome using short-read sequencing (DNase I hypersensitive site sequencing (DNase-seq))[45,46] (FIG. 2a). Boyle et al.[45] used a type II restriction enzyme to isolate and subsequently barcode each DNase cut site (single cut), whereas Hesselberth et al.[46] applied strict size selection to enrich for sequenceable fragments arising from paired cleavage events within DHSs (double cut) (FIG. 2a). Although there is broad agreement between these sequencing approaches, the Boyle protocol may identify more accessible locations, whereas the Hesselberth protocol provides a simplified workflow and captures fewer fragments that originate within broadly inaccessible chromatin (a higher signal-to-noise ratio). Collectively, these genome-scale chromatin accessibility measurements show that a minority of DHSs is found within promoters and transcription start site (TSS)-proximal regions, with over 80% of accessible regions resident within distal enhancers[13,43,45–47].

ATAC-seq. Assay for transposase-accessible chromatin using sequencing (ATAC-seq) uses a hyperactive Tn5 transposase to insert Illumina sequencing adaptors into accessible chromatin regions (FIG. 2b). Similar to double-cut DNase-seq protocols[13,25,47], ATAC-seq selectively amplifies proximal double-cleavage events in accessible chromatin. ATAC-seq measurements of accessibility are highly correlated with both double-cut ($r > 0.8$) and single-cut ($r > 0.75$) DNase-seq assays[29,33], although higher-resolution analyses — such as for TF footprinting[48,49] — can reveal differences in sequence bias. Owing to the high efficiency of Tn5-mediated adaptor ligation, highly complex ATAC-seq libraries have been generated with as few as 500 cells [29,33]. Variations of the ATAC-seq method have recently been described, including a report by Sos et al.[50] demonstrating library construction using in vitro transcription from single transpositional events. ATAC-seq has been widely adopted in part because it robustly identifies accessible chromatin, is straightforward and rapidly

Nucleosome occupancy
The fraction of time that a particular sequence of DNA is bound by the core histone octamer.

Epigenetic canalization
A set of persistent epigenetic features (alternatively, the process of establishing this feature set) that molecularly defines a cell type and comprises a continuum of cellular states including cell cycle phases and activation states.

TF footprinting
High-resolution analysis of chromatin accessibility data to identify a local accessibility signature in the neighbourhood of putative binding sites for a particular transcription factor (TF). This signature reflects the size and binding mechanism, as well as other biophysical properties, of a TF.

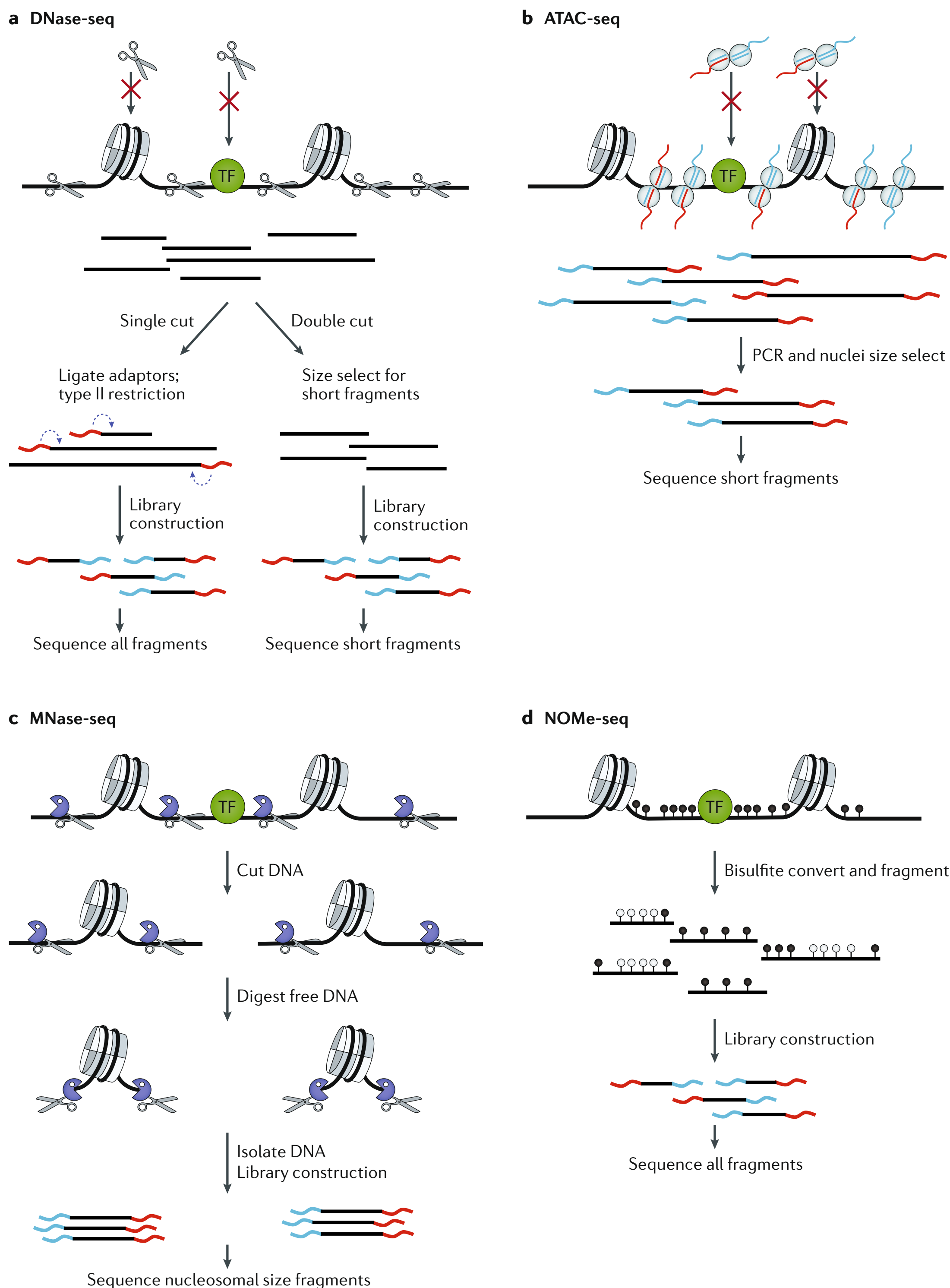

Fig. 2 | **Principal methods for measuring chromatin accessibility. a** | DNase I hypersensitive site sequencing (DNase-seq) uses the endonuclease DNase to cleave DNA within accessible chromatin. Endonuclease cleavage is greatly attenuated at protein-bound loci (the red crosses denote cleavage blockade). Accessible library fragments are generated by barcoding each cleavage site independently after restriction digest (single cut) or as proximal cleavage pairs (double cut). **b** | Assay for transposase-accessible chromatin using sequencing (ATAC-seq) uses a hyperactive transposase (Tn5) to simultaneously cleave and ligate adaptors to accessible DNA. **c** | Micrococcal nuclease sequencing (MNase-seq) uses the endonuclease/exonuclease MNase to both cleave and eliminate accessible DNA. The sensitivity to MNase digestion can be used to quantify chromatin accessibility in the MNase accessibility sequencing (MACC-seq) method. **d** | Nucleosome occupancy and methylome sequencing (NOMe-seq) uses a GpC methyltransferase to methylate accessible DNA. DNA sequencing following bisulfite conversion of nonmethylated cytosine to uracil nucleotides provides a single-molecule measure of accessibility. TF, transcription factor.

implemented and is amenable to materially limited clinical and primary tissue samples. Notably, ATAC-seq libraries are routinely generated in less than 2 hours with 10,000–20,000 cells; in comparison, DNase-seq is typically performed as a multiday protocol with hundreds of thousands of cells. Although the local sequence bias is known to differ between the two methods[51,52] (see REF.[53] for an excellent review describeing how to address DNase-seq and ATAC-seq biases), ATAC-seq and DNase-seq capture similar regulatory information[33,54].

MNase-seq. Given the central role of histone proteins in regulating chromatin accessibility, the nucleosome occupancy and positioning technique micrococcal nuclease sequencing (MNase-seq) has recently been adapted to measure accessibility[55,56] (FIG. 2c). MNase acts both as an endonuclease to cleave internucleosomal DNA and as an exonuclease to degrade cleavage-product DNA not protected by proteins. A notable difference between MNase-seq and both DNase-seq and ATAC-seq is the absence of nucleosomal DNA cleavage events. In fact, MNase probably cleaves nucleosomal DNA through its endonuclease activity; however, the evidence for these cleavage events is lost through exonuclease-mediated fragment degradation. As nucleosomal DNA is cleaved less efficiently by MNase than internucleosomal DNA, the enzyme has been widely used to isolate fragments that span single nucleosomes[57–59]. Drawing on prior work showing dose-dependent sensitivity of some nucleosomes to MNase[60], Mieczkowski and colleagues performed MNase-seq with different concentrations of nuclease to measure differential accessibilities across the genome[55,56]. This technique, termed MNase accessibility (MACC), relies on the observation that the representation of accessible DNA within an MNase-seq library decreases with nuclease concentration (likely owing to exonuclease-mediated digestion) while protected, nucleosomal DNA increases in relative abundance owing to the increased incidence of endonuclease-mediated cleavage of nucleosomal DNA. MACC signals are highly enriched at putatively active regulatory regions (regions positive for histone H3 lysine 27 acetylation (H3K27Ac)) and correlates well with DNase hypersensitivity data at TSSs, promoters and distal enhancers but not at transcription termination sites or within gene bodies[55,56]. This discrepancy may be explained either by differential enrichment of fragment lengths across the methods or by differences in the size of the enzymatic probes used.

NOMe-seq. Nucleosome occupancy and methylome sequencing (NOMe-seq) uses the GpC methyltransferase (MTase) from M.CviPI to probe chromatin accessibility[23,61] by chemically modifying, rather than cleaving, accessible DNA (FIG. 2d). This de novo MTase generates ectopic methylation at GC dinucleotides orthogonal to endogenous methylation at CG dinucleotides that is common in both human and mouse genomes. Thus, NOMe-seq simultaneously probes accessibility and the methylation status of DNA at high resolution because there is a high frequency of GpC throughout the genome. Importantly, NOMe-seq is not an enrichment method and therefore requires a comparatively large number of sequencing reads to obtain sufficient depth to determine accessibility levels over the whole genome. However, the absence of enrichment bias and the single-molecule character of this technique create a more quantitative view of the chromatin accessibility landscape than DNase-seq, ATAC-seq or MNase-seq, as the relative accessibility level of each genomic locus can be directly determined[23].

Single-cell accessibility methods. Measurements of single-cell accessibility variation hold promise to shed light on a central question of genomic regulation: how are short timescale fluctuations and developmental changes in chromatin accessibility coordinated across the genome? A variety of approaches have been developed for measuring chromatin accessibility in single cells on the basis of ATAC-seq, DNase-seq and NOMe-seq library preparation schemes.

Combinatorial indexing provides an elegant strategy to barcode thousands of single-cell ATAC-seq libraries[10]. In this approach, multiple transposition reactions are performed on purified nuclei with uniquely barcoded Tn5 enzymes and then pooled and split at limiting dilutions into multiple indexing PCR reactions. This approach exploits the low probability that any pair of cells sharing the same transposition reaction will co-segregate during the subsequent pool and split operation and thus does not require isolation of single cells. Combinatorial indexing has been used to profile accessibility across hundreds of thousands of single cells to profile embryonic development in *D. melanogaster*[62], build an atlas of murine cell types across 13 different tissues[63] and study transcriptional regulation in the developing mouse forebrain[64].

Single-cell ATAC-seq has also been implemented by capturing single cells within a microfluidic device (Fluidigm, C1)[8] or within individually indexable wells of a nano-well array (Takara Bio, ICELL8)[65]. Microfluidic capture has been used to profile accessibility across thousands of single cells during early haematopoiesis[30,31], whereas the nano-well arrays promise to further increase the throughput of microfluidic methods by an order of magnitude. Comparing these two platforms to the combinatorial indexing approach reflects a natural trade-off: whereas fewer cells are processed per experiment with microfluidic and nano-well array platforms, the single-cell library complexity is at least twofold higher[30–32]. The difference in library complexity is a critical parameter given the sparse sampling of even ubiquitously open regions (on average, only 10% of all open regions are observed in single cells using the C1 platform[31]), and we anticipate improvements in library complexity for both approaches in the near term. Single-cell ATAC-seq has recently been implemented on droplet-based microfluidic platforms from both 10X Genomics and Bio-Rad Laboratories. Early reports suggest that these high-throughput platforms will provide similar data quality to that of nano-well capture technologies.

Transposition-mediated single-cell accessibility profiling has quickly been followed by single-cell

adaptations of both DNase-seq and NOMe-seq[35,66]. Combining methylation, accessibility and nucleosome phasing in a proof-of-principle, single-cell NOMe-seq[34] assay, Pott reports 5.2–9.5% recovery of DHSs from single cells. Single-cell DNase-seq, although not readily scalable in its present implementation, generates single-cell accessibility fragment libraries with remarkable complexities of nearly 100,000 unique reads within open chromatin. Although the fraction of reads at DHSs is slightly lower than that of ATAC-seq-based methods[31,32] (23–26% for single-cell DNase-seq versus 35–55% for single-cell ATAC-seq), the number of unique fragments observed at DHSs remains relatively high. Notably, for a small fraction of DHSs, accessible fragments are recovered from these regions in nearly all single cells; furthermore, a typical DNase-seq library from a single cell contains fragments from 35–59% of all DHSs (compared with 10% for the single-cell ATAC-seq). Collectively, these proof-of-principle methods provide important research directions for building a single-cell epigenetic toolkit.

Interpreting chromatin accessibility profiles. Recent single-molecule and single-cell measurements of accessibility suggest that accessibility measurements on cell populations represent an ensemble average of distinct molecular states[23,31,32] (FIG. 3). This molecular heterogeneity arises naturally when observing an underlying, dynamic, unsynchronized process. At sites of transcription, for example, RNA polymerase — together with other TFs — displaces nucleosomes upstream of the TSS and migrates past well-positioned yet unstable nucleosomes downstream of the promoter (FIG. 3a). Footprinting analysis of single-molecule NOMe-seq data reveals snapshots of this process, capturing each molecular state as the transcriptional process unfolds (FIG. 3b). Although confounded by sampling efficiency considerations, single-cell measurements of accessibility capture similar molecular heterogeneity at predicted regulatory regions across the genome (FIG. 3c).

DNase-seq and ATAC-seq measurements of open chromatin in bulk cell populations can be viewed as proxies for aggregate TF-binding signals[13]. Bound TFs protect DNA from enzymatic cleavage, and the level of protection is related to the occupancy of TF binding on the DNA. We might likewise expect, given their relatively rapid off rates, that TFs generally provide less protection than nucleosomes. This subtle TF-associated protection requires the use of different bioinformatics analysis methods that leverage genome-wide prior knowledge of TF-binding affinity and cleavage-enzyme-specific sequence biases to interrogate TF binding and chromatin architecture (for an in-depth review of DNA footprinting, see Sung et al.[67])[52,68–71]. Combining accessibility with transcriptional data further extends this analysis stream: given a set of differentially accessible regions between cell types, developmental stages or other experimental conditions, TFs that are potentially involved in regulating the observed epigenetic differences can be identified by asking which TFs are differentially expressed and also putatively bind to differentially accessible DNA[69]. This remains an active area of research, and we anticipate substantial focus in the coming years on computational schemes that integrate accessibility with other epigenetic measurements.

Chromatin accessibility has recently been leveraged to identify causal genetic variants arising from genome-wide association studies (GWAS). Most single-nucleotide polymorphisms (SNPs) in the human genome occur in non-coding and intergenic genomic regions and individually contribute small or indirect effects to complex traits, thus confounding causal variant detection[72]. Recently, several studies have used chromatin accessibility to better understand these non-coding SNPs and their effect on traits[73–75]. Maurano et al.[74], for example, show that many causal GWAS variants are concentrated in non-coding DHSs and can be more accurately linked to the genes they influence by using chromatin accessibility data. These results underline the value of integrating chromatin accessibility data with functional and other epigenetic data analyses.

Biophysical determinants of accessibility

Physical access to chromatinized DNA is regulated at different length scales within the eukaryotic genome and is largely determined by the organization and occupancy of nucleosomes and DNA-associated macromolecules, including TFs and architectural proteins. DNA is almost universally bound by either histones or other DNA-binding factors[13,23,25], and competition for DNA by nonrepressive factors tends to increase accessibility at nucleosome length scales in spite of the fact that these factors locally occlude DNA binding at TF length scales. The reason that TF binding is correlated with accessibility is probably due to a variety of causes. First, the footprint of DNA binding proteins — including TFs and polymerases — is typically far smaller than the ~146 bp occupied by a nucleosome; consequently, non-histone protein binding makes substantially more DNA accessible than a fully constituted nucleosome does. Second, molecular interactions are fundamentally stochastic, and the average residence time of non-histone proteins in complex with DNA is often shorter than the timescale of nucleosome turnover[23,76,77], thus providing more frequent access to unbound DNA. Finally, sequence-dependent binding of proteins to DNA provides a recruitment substrate for nonspecific chromatin remodellers, which further open proximal chromatin, often by removing or repositioning nucleosomes. Given these factors, both the linear and topological organizations of histones have an outsized role in initiating access to DNA.

Nucleosome occupancy, density and turnover. Chromatin accessibility is influenced both by the density of associated proteins (particularly histones) along DNA and the fractional residence time of these factors. Perhaps counterintuitively, regions of high chromatin accessibility are distributed across a broad range of nucleosome turnover rates and occupancy levels, as illustrated in FIG. 4. For example, both CTCF-bound insulators and active TSSs are highly accessible, yet CTCF-bound insulators lie within regions populated by a dense array of stable nucleosomes, and TSSs are associated with unstable nucleosomes at low relative density.

Nucleosome turnover rates
The rates at which nucleosomes disassemble at particular genomic loci; alternatively, the inverse of the nucleosome residence times.

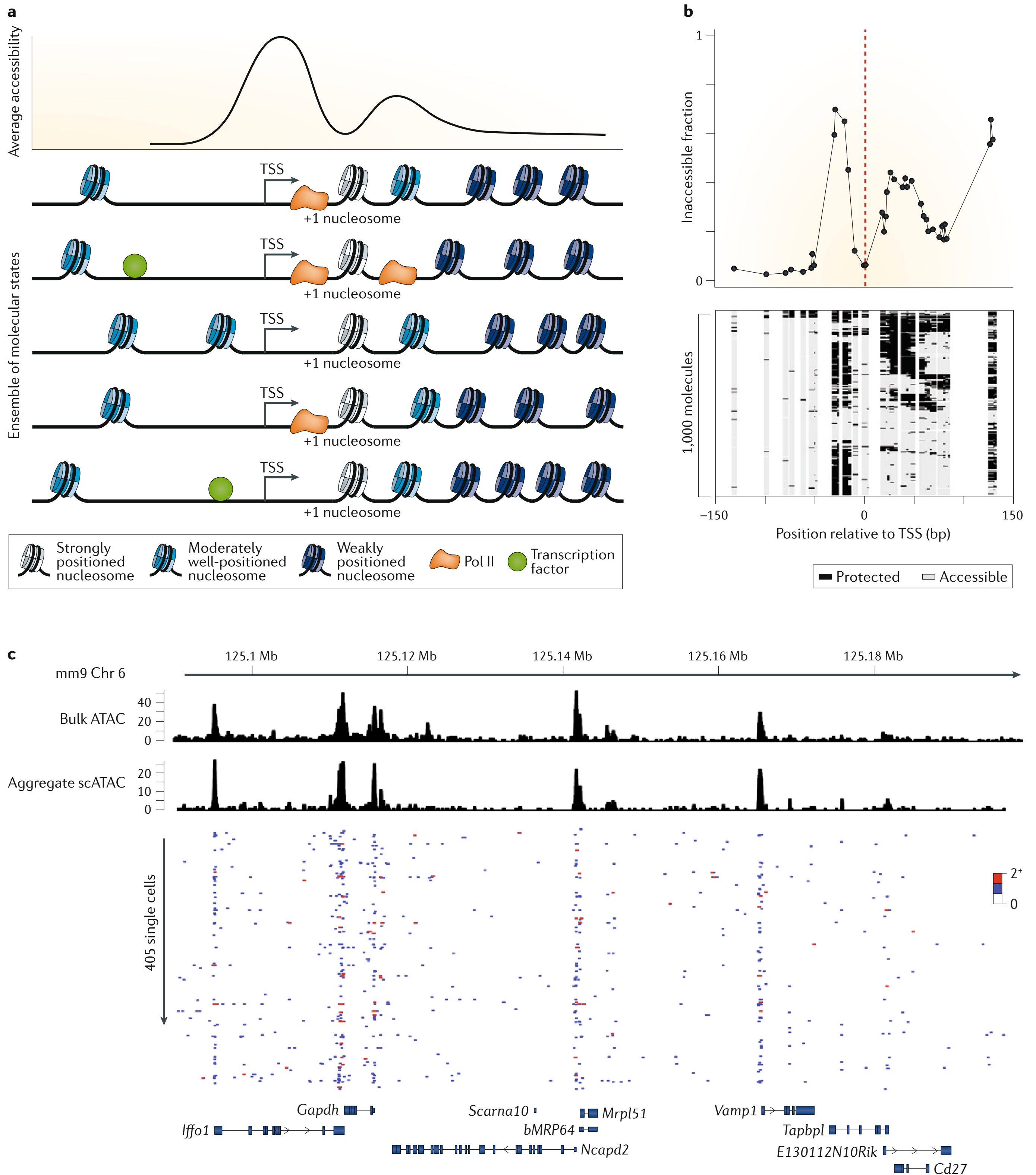

Fig. 3 | **Population-scale measurements of chromatin accessibility reflect the average accessibility of a heterogeneous collection of single molecules. a** | Accessibility of a transcriptionally active promoter and gene body shows that an ensemble of heterogeneous molecular configurations determines the population chromatin accessibility profile. **b** | Single-molecule measurements of accessibility (from nucleosome occupancy and methylome sequencing (NOMe-seq)) at transcription start sites (TSSs; red dashed line) reflect differential protection of DNA by bound proteins to methylation in vitro[23]. **c** | Single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) measurements of accessibility at a housekeeping gene locus show heterogeneous susceptibility of DNA to enzymatic cleavage by transposition[65]. Pol II, RNA polymerase II. Part **b** is adapted from REF.[23], Cell. Part **c** is adapted from REF.[65], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

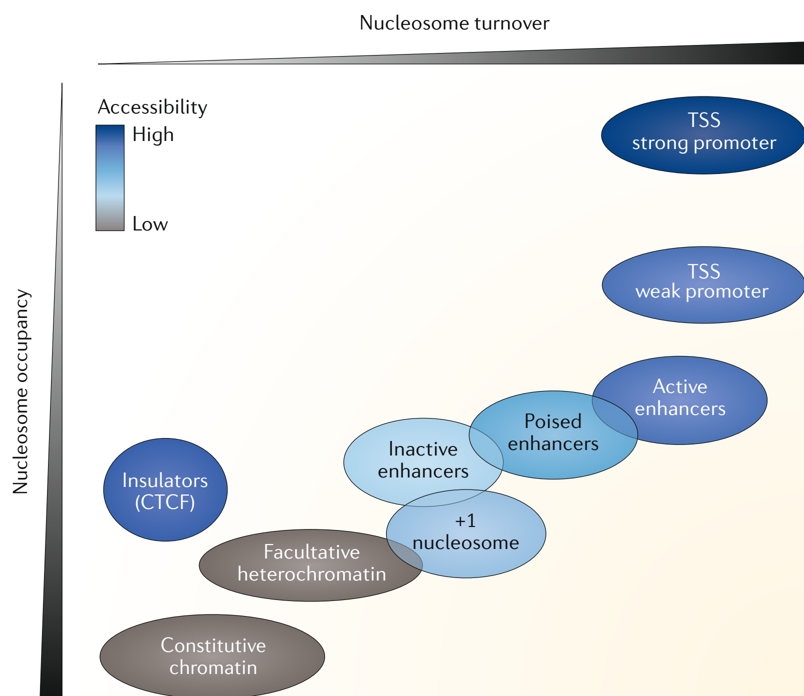

Fig. 4 | **Nucleosome turnover and occupancy are inversely correlated across a broad range of genomic regions.** Accessible chromatin is enriched within genomic regions of high nucleosome turnover, but it also coincides with dense nucleosome occupancy. TSS, transcription start site.

Both predicted active and poised enhancers occupy an intermediate position within the phase space of nucleosome occupancy and turnover, with nucleosome positioning and stability likely to be playing a dominant role in regulating DNA access by TFs[25,47,78,79]. This discordance between accessibility and chromatin dynamics contrasts with the simplified conceptual view illustrated in FIG. 1 and is likely to reflect the diversity of ways that accessibility functions in different chromatin contexts.

Nucleosome occupancy is dynamically regulated, with higher turnover rates in active promoters and enhancers than in inactive and heterochromatic regions[76,80–82]. For many promoters, two well-positioned nucleosomes upstream and downstream of the TSS define a nucleosome-depleted region[83] (NDR) (FIG. 3a). The size of the NDR is on the order of a single nucleosome but varies by activity and cell type, with expressed genes maintaining larger NDRs than inactive genes do[4,84–86]. The extent of nucleosome depletion at NDRs is a subject of active debate[87,88], yet an emerging consensus is that NDRs are infrequently occupied by nucleosomes[23]. Nucleosome exclusion within the NDR is maintained by a variety of factors, including BRG1/BRM-associated factor (BAF) and promoter-proximally paused RNA polymerase[89]. The formation of NDRs is not restricted to TSSs: depletion of nucleosomes that occlude access to regulatory DNA is commonly observed. For example, CTCF-bound insulators, RNA-polymerase-bound TSSs and glucocorticoid-receptor-bound enhancers demonstrate that high-occupancy TFs coupled with cofactor-dependent and ATP-dependent chromatin remodeller recruitment dynamically maintain nucleosome exclusion over short spatial length scales[25,52]. These structural motifs illustrate statistical nucleosome positioning[86,90,91] upstream and downstream of strong DNA–protein interactions.

Poised enhancers
Inactive enhancers that do not regulate gene expression but share a subset of epigenetic features commonly observed at active enhancers, including histone H3 lysine 4 monomethylation (H3K4me) and accessibility.

Nucleosome turnover rates are modulated by a variety of factors, including DNA sequence, histone variant composition, histone chaperone availability, the local chromatin context (comprising histone density and ATP-dependent chromatin remodeller activity) and occupancy by linker histones and TFs[4,92–97]. Nucleosome preference for particular DNA sequences is well established in vitro and in yeast[4]; however, this bias is less pronounced in multicellular systems[98], and it is weakly predictive of human nucleosome positioning[52,80,99]. Nucleosome positional diversity in higher-order eukaryotes may be due to the simple fact that multicellular systems require cell-type-specific nucleosome positioning patterns among cells that share DNA sequence. Non-canonical histone variants (commonly H2A.Z and H3.3) may also influence nucleosome turnover rates and are enriched in regulatory regions in the genome, including TSSs and enhancers[100,101]. Nucleosomes in these regions generally are less stable and contain post-translational modifications associated with rapid nucleosome turnover, such as histone acetylation and H3K4 methylation[102,103]. The localization of non-canonical histones within highly dynamic chromatin may be due to the differential availability of these variant subtypes during turnover events that are independent of replication. Functionally, high rates of non-canonical histone variant turnover at enhancers and promoters may provide the accessible substrate required for TFs to bind to and initiate chromatin remodelling in human cells[47,81].

Accessibility and linker histones. Another layer of chromatin regulation that influences chromatin accessibility is provided by linker histones and other architectural proteins that bind to the core histone particle near DNA exit junctions. Linker proteins play a crucial role in nucleosome positioning and heterochromatin formation[15,104,105]. Linker histones, including variants of histone H1, are generally understood to change the DNA nucleosome exit angle and to help neutralize the charge of linker DNA, thereby folding the chromatin into a more compact and less accessible fibre[16,19,106–109]. Recently, the high mobility group protein HMGD1 has been shown in *D. melanogaster* to displace H1 in open chromatin, including distal regulatory elements and active genes, with H1 being restricted to closed heterochromatin[17]. Although the impact of histone H1 depletion is phenotypically limited in cell culture[105,110–117], 50% H1 depletion results in embryonic lethality in mice and dramatic decondensation of constitutive heterochromatin throughout the organism[17,104]. Furthermore, H1 depletion has been linked to localized transcriptional changes, which may reflect the effect of linker-histone-mediated regulation of higher-order chromatin architecture on transcriptional initiation or elongation[104,118]. Notably, nucleosomal spacing is truncated in H1-depleted cells, suggesting that H1 is involved in maintaining nucleosome positioning. These observations indicate that histone H1 is likely to be a key player in structural maintenance of closed chromatin.

Accessibility, nucleosomes and the 3D genome. The linear structure of chromatin at the nucleosomal length scale is a primary determinant of chromatin accessibility; however, higher-order nucleosome organization may impact access to DNA as well[88,119]. Topological organization of chromatin at the 1 kb length scale — including chromatin fibre folding — has been shown in vitro to be influenced by two primary factors. First, core histone tail acetylation contributes to the decompaction of chromatin fibres[106,120] and is generally associated with accessible chromatin states[10]. Second, long linker DNA lengths and more regular nucleosome spacing is correlated with transcriptionally repressed, heterochromatic regions that are thought to be in a compacted state[95,121]. Chromatin fibre models that account for the torsional rigidity and helical geometry of linker DNA[94,122–124], as well as in vitro reconstitution experiments[19,125], show that fairly small changes in linker length contribute to considerable reorganization of the chromatin fibre both in terms of nucleosomal packing geometry and its response to supercoiling stress. In general, nucleosomal stacking within compacted chromatin in vitro is thought to reinforce uniform nucleosomal spacing and to inhibit chromatin accessibility[92].

Although not the focus of this Review, the regulatory role of higher-order chromatin structure in living cells is a subject of intense current interest. Recent ex vivo data have revealed differences in intrafibre nucleosomal packing across the genome, with more contacts between next-nearest-neighbour nucleosomes in heterochromatin than in euchromatin[126]. However, this difference may not be due to the presence of long-range order in highly regular chromatin fibres but rather due to locally ordered stretches of fibre, such as putative tetranucleosome units[127–129] or clusters of nucleosomes observed by super-resolution microscopy, which are smaller in euchromatin and larger in heterochromatin[107]. This view — that chromatin fibres are locally structured over short length-scales of a few nucleosomes — has been bolstered by direct observation of their conformation. Using electron microscopy, Ou and colleagues[130] directly visualized chromatin and showed that higher-order chromatin organization in vivo is disordered and does not fold into persistent 30 nm diameter chromatin fibres as observed in vitro, challenging the hypothesis that chromatin has a uniform structure beyond the nucleosome length scale. The authors argue that accessibility within living cells is principally determined by the concentration of chromatin rather than its folding geometry[130]. Further work will be required to resolve whether conformational features other than density and nucleosome contact frequency are important for regulating chromatin accessibility; however, this model is a notable departure from classical views of higher-order chromatin organization and may prove to be an important conceptual paradigm for understanding accessibility.

Accessibility remodelling

Physical access to chromatinized DNA changes dynamically in response to external stimuli[39] and developmental cues[131,132]. As chromatin accessibility is differentially regulated across the genome, TFs necessarily play a central organizing role in this process because other components of the remodelling machinery largely do not provide DNA sequence specificity. However, the overwhelming majority of surveyed TFs bind only to accessible, nucleosome-depleted DNA[13]; the canonical example of this biophysical restriction is the heat shock factor, which cannot bind to its cognate DNA target in the presence of core histone proteins but instead relies on the ubiquitous accessibility of the heat shock promoter for rapid induction of response genes[39]. Consequently, the mechanistic question of how TFs interface with closed chromatin to provide sequence-specific accessibility remodelling remains a subject of intense investigation. Here, we review several working models that explain how accessibility states are established and maintained by the coordinated action of DNA-templated TFs and active nucleosome remodellers (FIG. 5).

Passive competition between TFs and core histones. Arguably the most elementary and parsimonious model for accessibility remodelling proposes that TFs displace nucleosomes through passive competition for DNA binding[133,134]. In this mass action model, TFs gain access to histone-bound DNA by exploiting short periods of DNA accessibility during nucleosome turnover events (FIG. 5a). Local accessibility increases as the concentration of the histone-competing TF increases, providing opportunity for other TFs and cofactors to stabilize the accessible state. This mechanism is considered passive because it does not involve a direct interaction between TFs and nucleosomes; however, it is important to note that active chromatin remodellers modulate nucleosome turnover rates and are often involved in establishing a suitably permissive landscape for TF binding[135,136]. Naturally, this model is only viable within euchromatin because nucleosome turnover rates within heterochromatin are likely to be insufficient to support the requisite exchange. Higher-order histone packing within heterochromatin may additionally occlude TF access. The interpretation of accessibility under this model is that it is an inverse measure of the fraction of time that a particular genomic locus is bound by the histone octamer. Work by Svaren et al.[133] provides compelling evidence for this mechanism, showing that competitive DNA binding between TFs and histones is sufficient to explain TF-dependent exclusion of nucleosomes by C/EBPβ at distal regulatory regions. Subsequent work by Di Stefano and colleagues[26] further supports this model, showing that upregulation of C/EBPα induces accessibility of pluripotency-related genes and primes cells for reprogramming into induced pluripotent stem cells (iPSCs). The binding mechanisms of the glucocorticoid receptor (GR), a TF released under hormone signalling, likewise illustrates a multi-step remodelling mechanism: although glucocorticoid receptors bind almost exclusively (more than 95%) to constitutively accessible chromatin, an important class of GR binding sites is contained within inaccessible DNA in unstimulated cells[25,47,137]. The transient accessibility of these loci during hormone stimulation suggests that remodelling events immediately downstream of signalling establish an accessibility landscape that is amenable for GR binding.

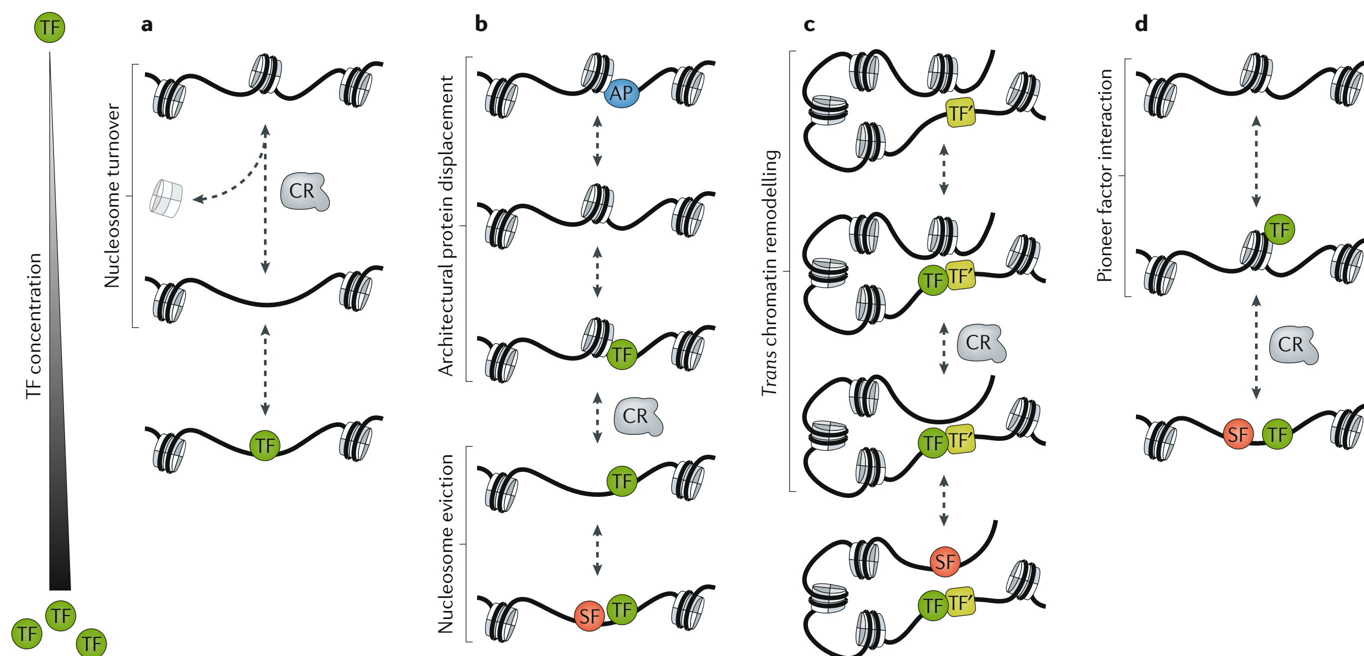

Fig. 5 | **Models of chromatin accessibility remodelling. a** | Transcription factors (TFs) initiate accessibility remodelling by passive competition with dynamic nucleosomes (shown in light grey). The turnover dynamics of nucleosomes are regulated by local DNA sequence, histone composition and specific chromatin remodeller (CR) activity. Passive competition for TF binding sites can involve nucleosome sliding or eviction (illustrated here). **b** | Together with active chromatin remodellers, TFs displace architectural proteins (APs) bound transiently to non-nucleosomal templates to initiate nucleosome eviction in *cis*. Active nucleosome displacement is stabilized by secondary TFs (SFs) that bind to the newly accessible DNA. **c** | Activated TFs bind to constitutively accessible enhancers (here, maintained by binding of a different TF (TFʹ)) and mediate nucleosome eviction and accessibility remodelling in *trans* by recruiting active chromatin remodellers and stabilizing secondary TFs. **d** | Pioneer TFs bind to nucleosomal DNA and directly open chromatin either independently or collaboratively with active chromatin remodellers. Binding of secondary TFs following nucleosome eviction stabilizes the newly established accessibility state. These models — though not comprehensive — are likely to explain the overwhelming majority of chromatin accessibility initiation events.

Further evidence for passive competition is provided by metabolic histone-labelling experiments showing that nucleosomes in regulatory regions of the genome are highly dynamic, with turnover rates measured at 10–20 times per cell division[76,138]. Kinetic modelling provides additional support for this model, showing that closely spaced TF binding sites may cooperate synergistically to regulate nucleosome occupancy through passive competition with nucleosomes[28,139,140]. Passive competition is also consistent with a recent report by Denny et al.[69] showing the metastasis-associated TF NFIB binds to weakly accessible target sites in primary tumours. These sites are frequently but not exclusively bound by nucleosomes, but upregulation of NFIB increases accessibility at these sites and nearby regions both in vitro and in mouse tumour models; a simple explanation for this observation is that NFIB competes with occluding nucleosomes passively by mass action kinetics for access to regulatory sites. Together, these data establish a flexible regulatory template within euchromatin for competitive binding of TFs to regions that are almost always — but not exclusively — bound by histones.

Accessibility remodelling during cellular homeostasis and embryonic development provides further evidence for the passive-competition model. A notable example of homeostatic accessibility maintenance is the recent report by Ramachandran et al.[141] that shows that TFs and histones competitively bind to recently copied DNA. This result may explain cellular sensitivity to global histone abundance and the restriction of canonical histone expression to replicating cells. Further developmental evidence that TFs and nucleosomes dynamically compete for DNA access is the observation in *Xenopus laevis*[142] that the onset of zygotic gene activation (the point at which a zygote produces its own gene products to supplement maternally contributed RNA and proteins) is principally determined by the stoichiometry of histones and DNA: zygotic transcription requires passive dilution of maternally contributed histones relative to the replicating genomic template. More directly, Joseph et al.[143] showed that zygotic gene activation is mediated by competitive exclusion of histones by TFs. RNA polymerase provides a further example of passive competition: for many gene promoters, ablation of RNA-polymerase pausing results in a compensatory increase in promoter-proximal nucleosomes[89]. Together, these results argue that a dominant and parsimonious mechanism for accessibility remodelling is passive competition between TFs and histones for DNA. In this model, differentially expressed TFs act on a landscape of accessibility and locus-specific histone dynamics that has been established by other TFs as well as active chromatin remodellers.

Chromatin remodelling in cis through proximal linker histone displacement. A second mechanism of accessibility remodelling involves a multistep process: TFs first bind to internucleosomal DNA and destabilize proximal nucleosomes, subsequently establishing access to core-histone-bound DNA though binding of stabilizing TFs (FIG. 5b). Destabilizing the core histone particle may involve recruitment of active chromatin remodellers; however, TF binding itself is often sufficient to bias occupancy of neighbouring histones in favour of other passively competing TFs[139]. The opening phase of this model relies on internucleosomal TF binding and often requires passive displacement of either linker histone H1 or other architectural proteins (for example, high mobility group proteins). In this context, architectural proteins broaden their structural functionality and may serve to protect DNA from nonspecific protein access while providing frequent access to DNA for specific TF interactions through their relatively high turnover rates[14,17,82,104,116,144–146]. In agreement with this model, evidence that TFs passively compete with architectural proteins for access to DNA is supported by a substantial body of research[25,47,137,141,142,147,148]. The TF nuclear factor-κB (NF-κB) — which is often associated with dynamic changes in accessibility[79,147,149] — primarily binds to open chromatin and is greatly attenuated at motifs localized within nucleosomal DNA[147,150,151]. These results suggest that passive competition, rather than active nucleosomal binding, may be the primary mechanism of action for this factor. When binding to closed chromatin, NF-κB has been shown to preferentially bind to linker rather than nucleosomal DNA in vitro[147,148], suggesting that NF-κB may initiate accessibility remodelling by binding to transiently accessible linker DNA. Further evidence in the context of progesterone signalling suggests that rapid and widespread displacement of histone H1 precedes ATP-dependent nucleosome remodelling[152]. Collectively, these observations argue that competition with linker-associated protein elements such as histone H1 and the high mobility group protein HMGD1 may provide a simple stepwise scheme whereby the binding of a single TF to a dynamic DNA element can destabilize a nucleosome, setting up the binding of a second *trans* factor that can replace the nucleosome itself.

Chromatin remodelling in trans through accessible, distal regulatory elements. A third model of chromatin remodelling is for a TF to bind to an accessible regulatory element and initiate distal chromatin remodelling in *trans*. This model is illustrated in Fig. 5c, in which a distally bound TF maintains an accessible binding site for an induced TF that recruits other cofactors to evict nucleosomes in *trans*. Direct evidence for this mechanism of accessibility remodelling is provided by Taberlay et al.[153], who show that TF recruitment to constitutively permissive enhancers initiates opening of Polycomb-silenced target promoters during myoblast reprogramming. This observation suggests that accessibility remodelling can be orchestrated in *trans* by TF recruitment to distal regulatory domains[153,154].

Direct binding of pioneer TFs to nucleosomal DNA. A fourth mechanism for accessibility remodelling argues that a class of pioneer TFs — including PU.1, members of the FOXA (also known as HNF3) family and GAL4 — directly bind to nucleosomal DNA to establish the open chromatin state[133,148,149,155–158] (FIG. 5d). The precise mechanism by which pioneer factors engage chromatin in vivo is not well understood and widely debated, in part because of disagreement over the definition of these factors. Historically, a pioneer factor is defined mechanistically as a TF that is capable of binding to nucleosomal DNA and independently producing an accessible state[149]; however, it is now commonly used as a functional category to describe TFs that are the first to bind to DNA during an accessibility remodelling process[71,157]. Many TFs satisfy the latter condition[71], but most of these are likely to mediate accessibility remodelling through one of the mechanisms outlined above. Irrespective of these semantic considerations, direct nucleosomal binding by pioneer TFs reflects a distinct regulatory mode, at least for in vitro reconstituted chromatin[157,159].

Whereas the majority of TFs bind to accessible DNA[13], pioneer factors arguably bind to nucleosomes and mediate core histone displacement either independently or by recruiting ATP-dependent chromatin remodellers[157,158]. There is clear evidence from in vitro reconstituted nucleosomal arrays that some TFs show sequence-dependent and concentration-dependent association with nucleosomal DNA in vitro[134,148,149,155–157]. However, conclusive experimental evidence for active accessibility remodelling by pioneer factors in vivo has remained elusive, in large part because the ternary complex composed of DNA, a TF and the core histone proteins has not been visualized in living cells. One line of indirect evidence for nucleosome binding, however, can be drawn from accessibility data: a fraction of subnucleosome-length DNase-seq and ATAC-seq fragments comes from cleavage events on nucleosomal DNA[29,45], reflecting a characteristic length distribution due to periodic (~10 bp) exposure of the double helix as it twists along the core histone particle. Importantly, this pattern is enriched in nucleosome-bound relative to nucleosome-depleted genomic regions[45]. This signal demonstrates the feasibility for enzymes, and thus potentially TFs, to productively interact with histone-bound DNA, probably during transient ‘breathing’ of these contacts. Collectively, these observations provide support for the feasibility of the classical pioneering model of remodelling.

Although the pioneering mechanism may play some role in accessibility remodelling, it is important to consider alternative, often more parsimonious, models in which pioneer factors bind to transiently accessible DNA rather than truly histone-bound DNA[16,118,147]. For example, the canonical pioneer factor FOXA1 — which plays a central role in remodelling accessibility across a broad range of developmental systems[149,157,158,160] — has recently been shown to displace linker histones (probably by passive competition) rather than to actively remove the core histone particle through templated binding of histone-bound DNA[161]. This raises the intriguing hypothesis that FOXA1 initiates accessibility

remodelling within closed enhancer regions by binding to internucleosomal DNA and recruiting active chromatin remodellers (FIG. 5b). Further evidence challenging the independent action of pioneer factors has emerged from an important study of the canonical pioneer factor PU.1 (REF.[27]); this TF binds to only a minority of its putative target sites, with many occluded targets enriched for highly stable nucleosomes[27]. This observation is readily explicable within a passive-competition model in which low nucleosomal turnover limits TF binding; however, if PU.1 has the capacity in vivo to bind to nucleosomal DNA (as the classical pioneering model argues[157,158]), its selective exclusion from these loci cannot be fully explained at the level of primary chromatin organization. Similar passive target selection has recently been reported for other pioneer factors, including FOXA2, GATA4 and OCT4 (REFS[159,162]). Overall, these observations suggest that pioneer factor binding is likely to be context-dependent[159] and requires a particular chromatin landscape to effect remodelling. From this perspective, one may consider many pioneer-like factors to be facultative TFs that initiate accessibility remodelling within broadly, yet not completely, inaccessible chromatin.

A diverse set of regulatory mechanisms for a heterogeneous chromatin landscape. The interplay between TFs and chromatin remodelling lies at the heart of gene regulation and remains an active area of research. Here, we have described four accessibility remodelling mechanisms that share common elements but are sufficiently distinct to capture the diversity of ways in which accessibility changes are likely to be initiated. For illustrative purposes, we have described the process through which each of these mechanisms can be executed in response to a concentration change in a single TF, although we expect that in practice, chromatin remodelling will involve coordination with many chromatin remodellers. The first two models outlined above (FIG. 5a,b) describe how accessibility states are established in *cis* for genomic loci where histone and architectural protein turnover rates are sufficiently high to afford transient access to DNA. These models capture a dominant mode of interaction between chromatin and TFs that passively compete with either the core histone complex or internucleosomal architectural proteins for access to DNA. The latter two models (FIG. 5c,d) illustrate how stable and well-positioned nucleosomes can be evicted either through *trans* interactions with accessible enhancer elements or direct binding of TFs to nucleosomal DNA. Notably, each of the models described here relies on an intrinsically dynamic organization of chromatin to initiate chromatin remodelling.

Conclusions and future directions

Chromatin accessibility is an analogue property of the physical genome that is established through a dynamic interplay among histones, TFs and active chromatin remodellers. Nucleosome occupancy and positioning are principal determinants of accessibility and are differentially regulated by sequence-specific TFs and chromatin remodellers, which are thought to bias the occupancy landscape of nucleosomes by modulating their assembly and eviction rates. This interplay is naturally achieved either through direct competition for nucleosomal DNA by subhistone-scale TFs or by recruitment of active chromatin remodellers that dynamically evict nucleosomes. This suggests that chromatin accessibility may be broadly interpreted as proportional to the average fraction of DNA molecules not occluded by histones or other macromolecules. We anticipate that new single-molecule and single-cell approaches will inform the biophysical and functional implications of this model in the coming years.

The relationship between the physical and functional genome is not well understood. When the linear structure of chromatin was first discovered, it was initially thought that nucleosomes would be excluded from transcriptionally active loci. However, early work by Felsenfeld and others challenged this hypothesis by showing that nucleosomes occupy actively transcribing gene bodies and are dynamically repositioned during transcriptional elongation by RNA polymerase[163,164]. Genome-wide analysis of accessibility and nucleosome occupancy has extended this work, arguing that enhancer activation is largely controlled by short-length-scale nucleosome dynamics rather than broad changes in occupancy[55,56,78,81]. Furthermore, although TSS-proximal nucleosomes often repress transcription[5], if properly positioned, nucleosomes may promote transcriptional elongation by RNA polymerase[165]. Together, these results suggest that the nucleosome is not simply a repressive unit but a central component of the regulatory landscape of chromatin.

Another illustration of the complex interplay between chromatin state and function relates to the transcriptional impact of enhancer and promoter accessibility; although both are necessary for transcription[39,166] and correlated with activity[13,29,45], inactive enhancers and promoters of transcriptionally silent genes are often open, suggesting that chromatin accessibility is necessary, although not sufficient, for enhancer or promoter activity[30,167]. These poised enhancers and promoters are accessible yet lack definitive marks of active regulatory regions, including enhancer RNA (eRNA) generation and histone acetylation[25,100,168,169]. Another example of discordance between accessibility and transcriptional activity is illustrated during zygotic gene activation in developing *D. melanogaster*: although widespread opening of chromatin is observed in the early embryo before activation, many accessible genes are transcriptionally inactive until later developmental stages[170]. Similarly, chromatin accessibility within regulatory genomic regions is more highly correlated with poised (for example, H3K4me1) than active (for example, H3K27Ac) chromatin states[131] during haematopoiesis. These data argue for a multilayered regulatory programme in which accessibility plays a broad licensing role but does not determine the activity state.

Until recently, clinical samples have largely been refractory to epigenetic analysis owing to prodigious material requirements. However, advances in small-scale chromatin accessibility assays promise broad clinical impact, particularly for understanding complex disease

states such as haematopoietic oncogenesis and immune exhaustion[171–174]. Recent work on cutaneous T cell lymphoma (CTCL) has revealed both the accessibility signature of oncogenic dysregulation and the epigenetic implications of therapeutic intervention[175]. Integrating epigenetic and transcriptional measurements in single cells — including recent reports of transcription and chromatin accessibility measurements in single cells[176,177] — will further broaden both our understanding of the epigenetic basis of disease and the clinical application space for epigenomics.

Chromatin accessibility represents a functional canalization of the epigenome by defining a repertoire of putative regulatory regions across the genome. The organization of open chromatin across enhancers, insulators, promoters and gene bodies provides a malleable, biophysical template though which components of the chromatin epigenome interact. These constraints establish physical interaction rules that functionally determine both transcription and cellular type, providing a powerful framework for epigenetically classifying cellular sub-states. While gene promoters are often constitutively accessible across a broad range of cell types, the accessibility of distal enhancers is often restricted by cell type. Understanding how these regulatory domains are dynamically established as cells transition between developmental stages and cellular activation states, as well as the biophysical rules governing how regulatory elements shape gene expression programmes, will be an important focal point of epigenetic research in the coming years.

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W.J.G. is a co-founder of Epinomics and an adviser to 10X Genomics, Guardant Health and Centrillion.

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