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Review

Rethinking chromatin accessibility: from compaction to dynamic interactions

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The genome is traditionally divided into condensed heterochromatin and open euchromatin. However, recent findings challenge this binary classification and the notion that chromatin condensation solely governs the accessibility of transcription factors (TFs) and, consequently, gene expression. Instead, chromatin accessibility is emerging as a factor-specific property that is influenced by multiple determinants. These include the mobility of the chromatin fiber, the capacity of TFs to engage repeatedly with it through multivalent interactions, and the four-dimensional organization of its surrounding diffusible space. Unraveling the molecular and biophysical principles that render a genomic target truly accessible remains a significant challenge, but innovative methods for locally perturbing chromatin, coupled with microscopy techniques that offer single-molecule sensitivity, provide an exciting experimental playground to test new hypotheses.

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Introduction

Flemming coined the word *chromatin* 'for the time being' in 1880 to designate the substance in the cell nucleus absorbing his aniline dyes [1]. Later, improving staining techniques, Heitz introduced the dichotomy between the gene-poor, condensed, silent heterochromatin and the gene-rich, open, active euchromatin [2]. Following studies on position-effect variegation [3] provided a crucial functional aspect to this simple model and explains its longevity. In this classical view, the condensation of heterochromatin prevents transcription, and genes can be exposed or isolated by moving them between these two compartments.

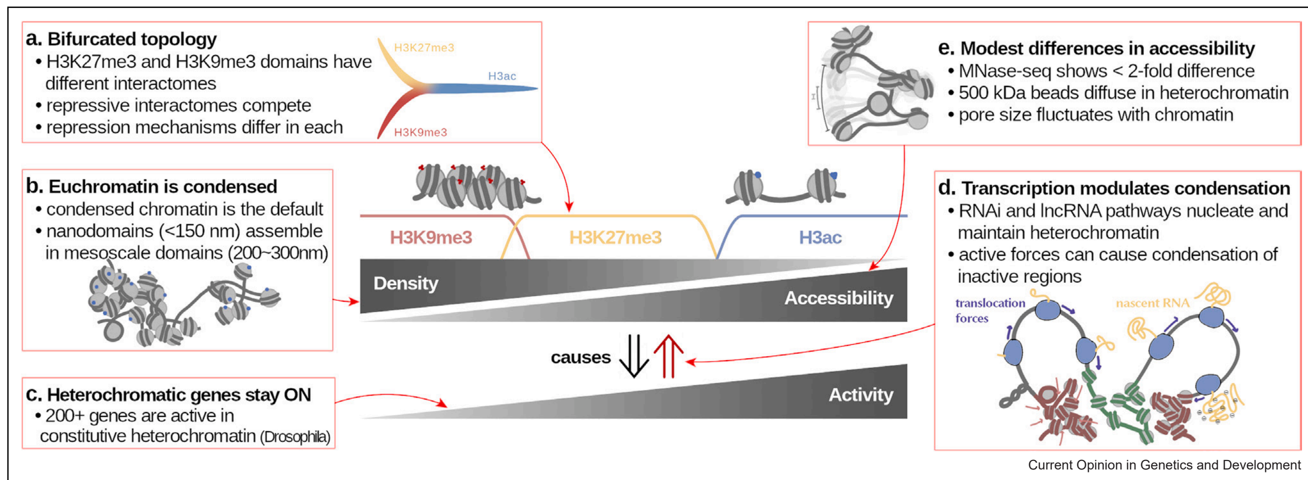
Advancements in microscopy and biochemistry are challenging this model. At the nanoscale, all chromatin appears organized into similarly condensed domains both in euchromatin and heterochromatin, leading some to question whether euchromatin is really open in the cell [4]. Furthermore, if condensation does not distinguish active and inactive chromatin compartment, what does? Here, we review recent literature highlighting phenomena other than condensation that might determine whether a genomic locus is accessible or inaccessible — ranging from the local mobility of the chromatin fiber to the 4D organization of the diffusible space around the chromatin locus and the capability of transcription factors (TFs) to repeatedly interact with the same locus due to multivalent interactions. Dissection of these processes calls for improved approaches to quantify dynamic events at specific genomic loci in the nucleus of living cells.

Multiple definitions for euchromatin/heterochromatin

A first challenge when discussing euchromatin/heterochromatin is that their definitions can vary depending on methods used to investigate them.

According to molecular biology, heterochromatin is characterized by specific histone post-translational modifications (PTMs): H3K9me3 or H3K27me3 marks heterochromatin, while euchromatin is generally more acetylated [5]. These modifications interact with reader and writer proteins, potentially leading to polymer phase separation [6,7]. The relation between chromatin modifications and chromatin 'state' is often represented as a linear one, with facultative heterochromatin, marked by H3K27me3, sitting in an intermediate state between H3K9me3-enriched constitutive heterochromatin and euchromatin. However, recent data point toward a bifurcated topology, with constitutive and facultative heterochromatin being characterized by distinct interactomes (Figure 1a) [8,9]. These possibly underlie why the histone 'code' can be nondeterministic, having context-dependent effects based on the local chromatin environment [10]. For example, H3K9me3 is associated

Figure 1



Classical description of eu/heterochromatin and associated challenges. **(a)** *Rather than following a linear continuum, chromatin states may occupy a bifurcated topology.* Proteomic shows that H3K27me3 and H3K9me3 recruit different interactomes based on reader/writer proteins [8,9] or DNA elements like transposons [12]. Those two repressive interactomes can compete for the recruitment of factors like PRC2 [7] or nuclear positioning [13]. Genes are repressed differently within each interactome: in some domains, loss of H3K9me3 leads to gene expression despite high H3K27me3 levels, indicating the H3K27me3 does not recruit its repressive interactome there [14]. **(b)** *Euchromatin is condensed*. Microscopy [15-17] shows that both euchromatin/heterochromatin organize in small aggregates of nucleosomes in irregular shapes. Heterochromatin domains are larger, yet the irregular shapes mean the fraction of chromatin exposed on the surface is roughly constant with size [17]. **(c)** *Heterochromatic genes are expressed in heterochromatin*. In *D. melanogaster*, a minimum of 230 protein-coding genes are located in constitutive heterochromatin [18]. Intriguingly, their expression is perturbed if moved away from pericentromeric heterochromatin [19]. **(d)** *Transcription can modulate chromatin architecture*. RNAi-dependent and independent pathways are necessary for establishment and maintenance of heterochromatin [20,21]. This requirement is continued, as transcription of major satellite repeats is required for proper mitosis in mouse embryos [22]. Inhibition of transcription leads to chromatin compaction [23,24], and modeling work shows differences in activity by itself can drive microphase separation [25]. **(e)** *Chromatin density limits transcription machinery accessibility, but only partially*. Controlled MNase-seq experiments show the difference in absolute accessibility between euchromatin/heterochromatin is less than twofold [26,27]. In live cell, dextran beads of 500 kDa and large macromolecules have been shown to be able to diffuse in heterochromatin regions [28,29], although there is conflicting evidence [30]. Accessibility of such large molecules may be due to the fluctuations of chromatin — and thus pore size — which are larger in less dense regions [31].

with transcription activation immediately after fertilization in mammalian embryos [11].

Chromosome conformation capture approaches such as Hi-C offer a different perspective. The analysis of principal components in Hi-C contact maps defines juxtaposed A/B compartments that roughly match with the euchromatin/heterochromatin dichotomy. At the > 10 kbp scales, enrichment of euchromatic marks is found in the A compartment, while the B compartment is enriched in repressive marks. Recent Hi-C data [32] however show that this correlation partially breaks down at finer (500 bp) scale: while enhancers and promoters are still found enriched in the ‘A’ compartments, most of ‘B’ compartments are uncorrelated with repression-associated PTMs at this scale, and chromatin often switches from A to B in the middle of active genes. Direct comparison across resolutions is not trivial: because they are defined by the variance in the contact matrices, A/B compartments are statistical constructs whose biological underpinning may shift with scales, depending on which phenomenon creates the most variance [33]. Additionally, bulk Hi-C averages over dynamic chromatin states — dynamics that are likely affected by the scale of the phenomenon, resulting in different distributions within the population [34]. Nonetheless, nested microcompartments identified at specific loci [35] suggest that microcompartments are relevant features of chromatin organization.

For microscopists, local density of DNA or fluorescently labeled histones remains the base criterion to discriminate between euchromatin/heterochromatin [15,30]. Gelléri et al. [30] recently provided quantitative measurement of DNA densities in mammalian nuclei, finding ranges from 5 to 300 $Mbp/\mu m^3$ and drew the line between active and inactive at 40 $Mbp/\mu m^3$. Yet, both electron [16,17] and super-resolution optical microscopy [15,36,31,37] reveal that chromatin organizes into domains of 100–1000 nucleosomes, and such organization is similar for both ‘open’ and ‘closed’ chromatin at the nanoscale (Figure 1b) [4]. Notably, while heterochromatin is more chromatin dense, it is only 1.5-fold more crowded overall than euchromatin because non-nucleosomal components dominate in both regions, suggesting only a moderate barrier to access [38].

Thus, at finer scales, the difference between euchromatin/heterochromatin blurs. A view that could reconcile nanoscopic observations of chromatin structure and difference in behavior of euchromatin/heterochromatin is one where domains are similar, but the diffusible space between them (i.e. their mesoscale packing) is different, impacting factor diffusion. This paradigm shift requires rethinking how chromatin controls gene expression in the absence of 'open' chromatin. A proposed solution is the 'buoy model' [4,39], positing that small TFs penetrate dense domains to bind specific loci and expose them on the domain surface, where they are anchored by large activator complexes. This model hinges on (1) *surface exposure*, where activation occurs by positioning loci at the domain surface, and (2) *size-dependent* exclusion, where dense chromatin restricts access to larger macromolecules.

Surface exposure is supported by the observation that active marks are enriched at chromatin domain surfaces [15]. On the other hand, Li et al. [17] measured a median exposure ratio of 25% for chromatin domains, and live-cell imaging of the chromatin fiber shows nucleosomes moving distances comparable to the domain size [36], suggesting that targets located in the repressive core could have some chance to be exposed on the surface due to stochastic fluctuations. The repressed core notion is also challenged by findings in *D. melanogaster*, where > 200 genes within constitutive heterochromatin lose expression when relocated away from pericentric heterochromatin [18,19] (Figure 1c). Additionally, transcription of heterochromatin is essential for its own establishment [20,21] and on an ongoing basis, as satellite repeat transcription is required for proper mitosis in mouse embryos [22]. Furthermore, transcription impacts chromatin structure itself: blocking transcription increases compaction [23,24], and modeling suggests polymerase activity drives chromatin microphase separation, condensing nearby non-transcribed regions [25]. Thus, the causality link between chromatin compaction and chromatin activity can point in both directions (Figure 1d).

Size-dependent exclusion is supported by the correlation between the radial histone mark distributions around dense chromatin domain and the size of chromatin modifier complexes responsible for these modifications [15]. Gelléri et al. [30] further suggested that chromatin domains have different pore sizes (24–1 nm) that limit access for larger macromolecules, as seen with the exclusion of 20–40 nm nanoparticles from the denser chromatin. Earlier studies, however, observed that macromolecules (including TFIIH) up to 90 nm can access chromatin-dense mesoscale domains [25,28] (Figure 1e), although this could be a consequence of their overexpression.

Chromatin accessibility can also be probed biochemically, with DNA-cleaving enzymes (MNase, DNase) or transposases (Tn5), which leave marks on the genome that can be recognized by sequencing. Accessibility measured in this way is therefore a convolution of the capability of the enzymes to explore a certain chromatin region and of the actual binding to the target region. Notably, at the molecular scale, both MNase and Tn5 enzymes are sensitive to masking of DNA by histones — as are other DNA-binding proteins — and retrieve similar nucleosome positioning. Yet, they provide different mesoscale pictures, Tn5 showing a much starker difference in accessibility between euchromatin/heterochromatin due to its stronger bias for nucleosome-depleted regions [40,26,27,41]. Interestingly, these differences in accessibility seem to be regulated by differences in interactions with chromatin, rather than in size: for example, fusing Tn5 with the chromodomain of HP1α has been shown to target H3K9me3-enriched heterochromatin, despite its increased size [42]. Thus, accessibility might also depend on specific interactions with chromatin features, hinting that the buoy model could be expanded to incorporate other properties beyond the size of the diffusible factor. These might include how chromatin moves and how it is explored by soluble factors, questions that can be targeted by live-cell imaging of single molecules in the nucleus [43].

What does it mean for chromatin to be accessible?

Single-molecule tracking (SMT) of nucleosomes (reviewed in Ref. [44]) has been used to gain insights into the dynamical aspect of chromatin architecture, beyond the snapshots offered by fixed-cell microscopy. Two major insights relevant to the current discussion emerged from those studies.

First, chromatin exhibits motion on a short scale, with displacements on the order of 100 nm within 0.5 s [36,31,45,46]. This mobility leads to fluctuation of the pore size of chromatin domains, which could partly explain how some large macromolecules access densely packed regions [47]. Notably, chromatin motion is different in regions at different density [37] and replication timing [48]. Furthermore, variable combination of both chromatin motion and confinement separates different types of chromatin and correlates with binding of distinct regulatory factors [46]. Along the same lines, Saxton et al. [49] used live-cell imaging of PTMs to show that even different flavors of euchromatin display distinct dynamic behaviors: chromatin enriched with H3K27Ac is spatially segregated from chromatin loaded with initiating PolII, and these two compartments display different nucleosome mobility.

Second, chromatin motion is correlated over short distances, typically < 150 nm [36,50]. Such correlations inform on the physical processes, leading to the formation of microcompartments, such as loop extrusion [35] and polymer

phase separation [51]. Both likely co-exist and compete to influence the organization of mesoscale domains [52–54], and mathematical modeling suggests they could affect chromatin motion differently [51], which might be reflected in the stronger correlation of movement in heterochromatin [48]. Furthermore, as different proteins and RNA species are involved in the multivalent interactions that cause segregation from bulk solution of different substrates, multiple flavors of phase separation in the nucleus exist [7,20,55–57]: these different phase-separated compartments could differently partition different subsets of soluble factors, leading to the possibility of 'compartment-specific' accessibility. While experimental and theoretical efforts to characterize multiphase coexistence and its robustness are a matter of active research [8,58,59], recent modeling work shows that cross-linkers affect the movement of factors diffusing within the domain, impacting mean escape time, penetration length, and anomalous diffusion in the compartment [60]. In parallel, thus, studying the movement of soluble factors within chromatin provides information about the underlying properties of those domains and on the organization of the space they explore.

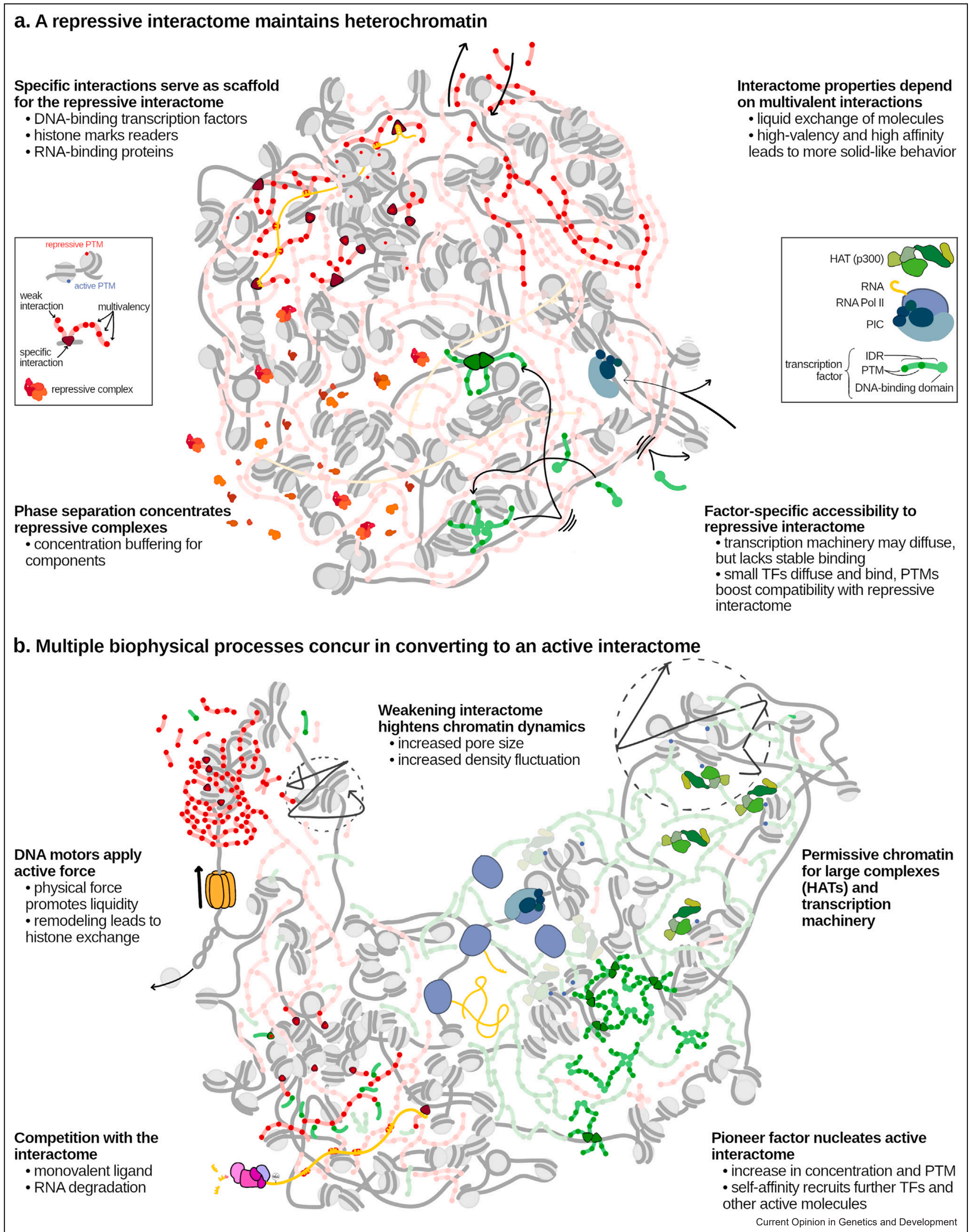
A first result is that different TFs navigate the nucleus in different ways. Generally speaking, exploration can be classified as compact or noncompact, where a compact exploration occurs when a molecule has a high probability to completely explore a region of space before leaving it (reviewed in Ref. [61]). While it is efficient if the target is close, compact search is also distance dependent: if the TF compactly explores a region poor of targets, it will take time to escape it. Anomalous diffusion and anisotropy are two signs of compact exploration, and SMT studies show that some molecules like MYC, p65, or inert tracers do not show much signs of compact exploration [62,63], while other TFs like CTCF, p53, FOXA1, and members of the preinitiation complex do [31,63–66]. Different molecular players might act as substrates for such compact exploration, ranging from RNA [64] to histone PTMs [67]. Given the nonrandom distribution of histone modifications in regions of varying chromatin density [15], this could lead to different TF behaviors in different chromatin contexts. In particular, pioneer factors tend to exhibit more compact exploration specifically in DNA dense regions [63,65] and may engage in guided exploration — alternating between compact and noncompact modes. This phenomenon manifests as peaks in anisotropy at specific distances, corresponding to the size of confinement zones where TFs are transiently trapped. Interestingly, compact exploration of 'closed' chromatin resulted in repeated interactions of the pioneer factor with the same targets and increased occupancy at those locations [65].

Similarly interesting is asking what features of the soluble factors can mediate their exploration strategy, with recent research converging on intrinsically disordered regions (IDRs) playing a major role [68,69], by interacting with each other and with the scaffold of the phase-separated compartments and other condensates [70]. Lerner et al. [46] showed that FOXA1 and SOX2 scan repressed chromatin differently: FOXA1 moves slowly with longer interactions; SOX2 moves rapidly with transient interactions. Imaging the DNA-binding domains on their own showed altered motion and reduced ChIP-seq peaks in DNase-resistant regions. Our group directly imaged DNA density at high resolution alongside SMT of various factors, confirming the role of p53's IDR in its motion within chromatin [63]. We showed that a p53 mutant lacking its IDR was less able to penetrate high-density chromatin regions. Similarly, Wang et al. [65] found that pioneer TFs rely on their IDRs to access compacted chromatin and efficiently target silent chromatin, essential for their gene regulatory functions. Both studies investigated IDR-specific behaviors by swapping protein IDRs. Fusing p53 to the FUS IDR reduced its enrichment in dense chromatin and decreased expression of its target genes, especially at high p53 concentrations where it was diverted into condensates [63]. In contrast, fusing the FOXA2 IDR onto FOXA3 or SOX2 increased their ability to penetrate high-density chromatin and resulted in higher pioneer activity [65]. Notably, the binding sites gained by SOX2 fused with the FOXA2 IDR were genuine SOX2 targets, not FOXA2 targets. These results underline that low-affinity multivalent interactions can mediate specificity, a result also confirmed when swapping IDRs across two different isoforms of the same TF [69].

Is accessibility a matter of competing interactomes?

Live-cell imaging data therefore highlight that accessibility and exploration of different chromatin compartments is a factor-specific property that often depends on low-affinity-mediated interactions between the IDR of diffusible factor and components within the compartment. These interactions influence accessibility on two distinct but related layers: the physical ability to diffuse into chromatin domains and the capacity for specific molecular reactions. For instance, the transposase Tn5 is blocked by histone masking of DNA — leading to its preference for nucleosome-free, hyperaccessible regions [40], and recent data show polycomb-mediated repression blocks not the diffusion of preinitiation complex (PIC) components, but their stable binding and assembly [71]. Maintaining chromatin in a silent state similarly depends on a network of specific and low-affinity multivalent interactions involving chromatin readers and writers with DNA [72] and RNAs [73]. This network forms a repressive interactome (Figure 2a) and promotes the assembly and function of repressive macromolecular

Figure 2



Generalized view of pioneering activity. **(a)** *A repressive interactome maintains heterochromatin.* Histone readers establish chromatin domains via specific interactions, like CBX and Ccc1, which in turn bring repressive complexes, such as polycomb, to repressive histone marks [7]. DNA-binding proteins like MeCP2 [72] and RNA-binding proteins like SAFB [73] also anchor to chromatin and interact either specifically or multivalently with the domain, creating repressive hubs at appropriate loci. The interactome's properties are shaped by protein concentration and the valency and affinity of interactions [74], and its viscoelastic properties may change overtime, affecting the mobility of diffusible factors [55]. Concentration buffering of compatible factors promotes the assembly of repressive condensates, overall favoring repressive interactions. Access of large complexes like the transcription machinery could be partially but not completely impeded, but the hostile interactome prevents stable binding [71]. For smaller TFs, pore size is not restrictive; however, their IDRs and associated PTMs can further influence their compatibility and promote guided exploration [63,65]. **(b)** *Multiple biophysical processes concur in converting to an active interactome.* Chromatin remodelers can act on the interactome in an energy-dependent manner. Applied forces can fluidify the condensate [75]. Monovalent ligands can disrupt the interactome by reducing condensate coherence [76], while long, multivalent molecules such as RNA significantly alter condensate density [77], making RNA a critical regulatory lever [78]. Pioneer TFs, at sufficient concentration and with appropriate PTMs can co-cluster at binding sites with DNA [79]. With adequate self-affinity, this initial clustering can out-compete the repressive interactome, nucleating a more active one. This chemical and physical disruption loosens the interactome, decompacting chromatin and increasing its fluctuations, which makes the domain more accessible to transcription machinery and HAT complexes that deposit active marks. Importantly, transcription itself applies physical forces and alters the electrostatic environment via negatively charged nascent RNA, which likely further impacts the biophysical properties of the active interactome [80].

complexes such as polycomb [7]. By shaping the structure and dynamics of chromatin, this interactome might limit the accessibility and/or function of activating complexes [71]. Pioneer TFs can nevertheless penetrate these domains and might have evolved IDRs capable of competing with the repressive interactome [46,65].

As the IDR of pioneers promotes repeated interactions with targets, this might enable high occupancy of 'inaccessible sites' at low TF concentration [81]. The genomic analysis of binding of native chromatin by TFs at varying concentration obtained by BANC-seq [82] reinforces this notion, showing that pioneer TFs like FOXA1 bind strong motifs independently of accessibility or concentration, while clusters of weak motifs — more likely to be near promoters — respond to increasing FOXA1 levels. This pattern aligns with the notion that repeated binding of pioneers might be important for their activity [81], making targets more accessible to other factors by modulating the biophysical properties of the repressive interactome, including its geometry and its dynamics. In agreement with this possibility, the modulation of the expression of the pioneer TF CDX2 has been shown to perturb chromatin dynamics across the nucleus, and this is associated with a reorganization of local chromatin contacts and of A/B compartments, as probed by HiC [50]. Similarly to the buoy model, once accessible the targets in the compartment could be bound by previously excluded macromolecular complexes, promoting a switch to a transcription-permissive interactome (Figure 2b) that could in turn prevent the target to be silenced back.

How could this model be tested? One first possible experiment would be to verify if local chromatin and diffusible factor mobilities change around selected specific targets upon their 'repositioning' into in different environments, for example, by combining long-term 3D SMT [67,83–85] with recent tools to exert forces on a locus by physical [86] or biochemical [87] means. The proposed model also predicts that repressed chromatin domains locally exposed to high concentration of activating factors and/or modifications, would switch to an activating state, together with their surroundings, and potentially in a bistable manner: combining tools to control the local epigenetic modifications at a specific genetic locus in a tunable manner [88], with live-cell measurement of accumulation of histone modifications at the same site [49] for prolonged time could address this question. We hope that the rapid advances in biotechnology and microscopy approaches will soon allow us to experimentally challenge this and alternative models for transcriptional regulation.

CRediT authorship contribution statement

Conceptualization: TF, DM. Writing: TF, DM. Visualization: TF. Investigation: TF, DM. Supervision: DM. Funding acquisition: DM.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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