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Review

Understanding and Engineering Chromatin as a Dynamical System across Length and Timescales

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SUMMARY

Connecting the molecular structure and function of chromatin across length and timescales remains a grand challenge to understanding and engineering cellular behaviors. Across five orders of magnitude, dynamic processes constantly reshape chromatin structures, driving spaciotemporal patterns of gene expression and cell fate. Through the interplay of structure and function, the genome operates as a highly dynamic feedback control system. Recent experimental techniques have provided increasingly detailed data that revise and augment the relatively static, hierarchical view of genomic architecture with an understanding of how dynamic processes drive organization. Here, we review how novel technologies from sequencing, imaging, and synthetic biology refine our understanding of chromatin structure and function and enable chromatin engineering. Finally, we discuss opportunities to use these tools to enhance understanding of the dynamic interrelationship of chromatin structure and function.

The regulation of the human genome in three-dimensional space is staggeringly complex. The genome self-organizes over five orders of magnitude of space and time, with dynamic processes such as transcription, cellular division, and differentiation constantly reshaping structures (Phillips et al., 2012). Robust cellular function requires proteins to access genetic information in an ever-shifting landscape of structures. Chromatin (see Glossary), the chromosomal DNA polymer wrapped around nucleosomes, is dynamically remodeled during replication and transcription. Emergent hierarchies of actively folded structures may dynamically facilitate or impede access to different regions across these scales (Zhang et al., 2019). Understanding the mechanisms of chromatin organization is crucial to understanding how cells function (Figure 1).

With increasing resolution, the relatively static, hierarchical view of genomic architecture is being revised with an understanding of how dynamic processes drive organization across the breadth of length and timescales. The local and global state of chromatin is dynamically altered through a combination of induced forces, torques, and chemical alterations which both operate on, and bridge, the respective length and timescales of cellular function (see Box 1). These effects provide a scaffold for epigenetic memory and a channel for information transmission that must work collectively for the emergence of spatial and temporal fidelity of crucial cellular functions such as replication and transcription (Rivera-Mulia et al., 2019). Gaps remain in our understanding of how transcription and transcriptional regulation impact structures to enable the emergence of new transcriptional states. Within these gaps, there exist opportunities for new technologies and integrative approaches to bridge models between imaging and sequencing technologies, between static and dynamic observations, and between single-cell and bulk population analyses. Recent experimental developments have begun to bridge this divide, ushering in the opportunity to extract greater understanding of how organizational structures contribute to functional behavior across various scales.

Here, we provide an introduction to chromatin and the modes of chromatin regulation to acquaint new researchers in the field. With this background, we review emerging technologies in sequencing and imaging that are forging new models for how chromatin is dynamically regulated in space and time. Additionally, we examine how engineered chromatin systems, from synthetic transcription factors to synthetic contact domains, engage emerging questions in gene regulation and chromatin dynamics from a bottom-up approach. Finally, we present a discussion of the emerging questions in chromatin regulation and opportunities to deploy novel technologies to address these questions.

INTRODUCTION TO CHROMATIN AND DYNAMIC MODES OF GENE REGULATION

Chromatin can facilitate pattern formation and information transfer across multiple time and length scales (Figure 1A). Nanometer-scale interactions such as transcription factor binding occur on the order of milliseconds (Fierz and Poirier, 2019), while chromosome territories, the largest subnuclear structures, persist over many cell divisions (Dekker and Mirny, 2016). Because of the short-ranged stochastic nature of DNA-protein

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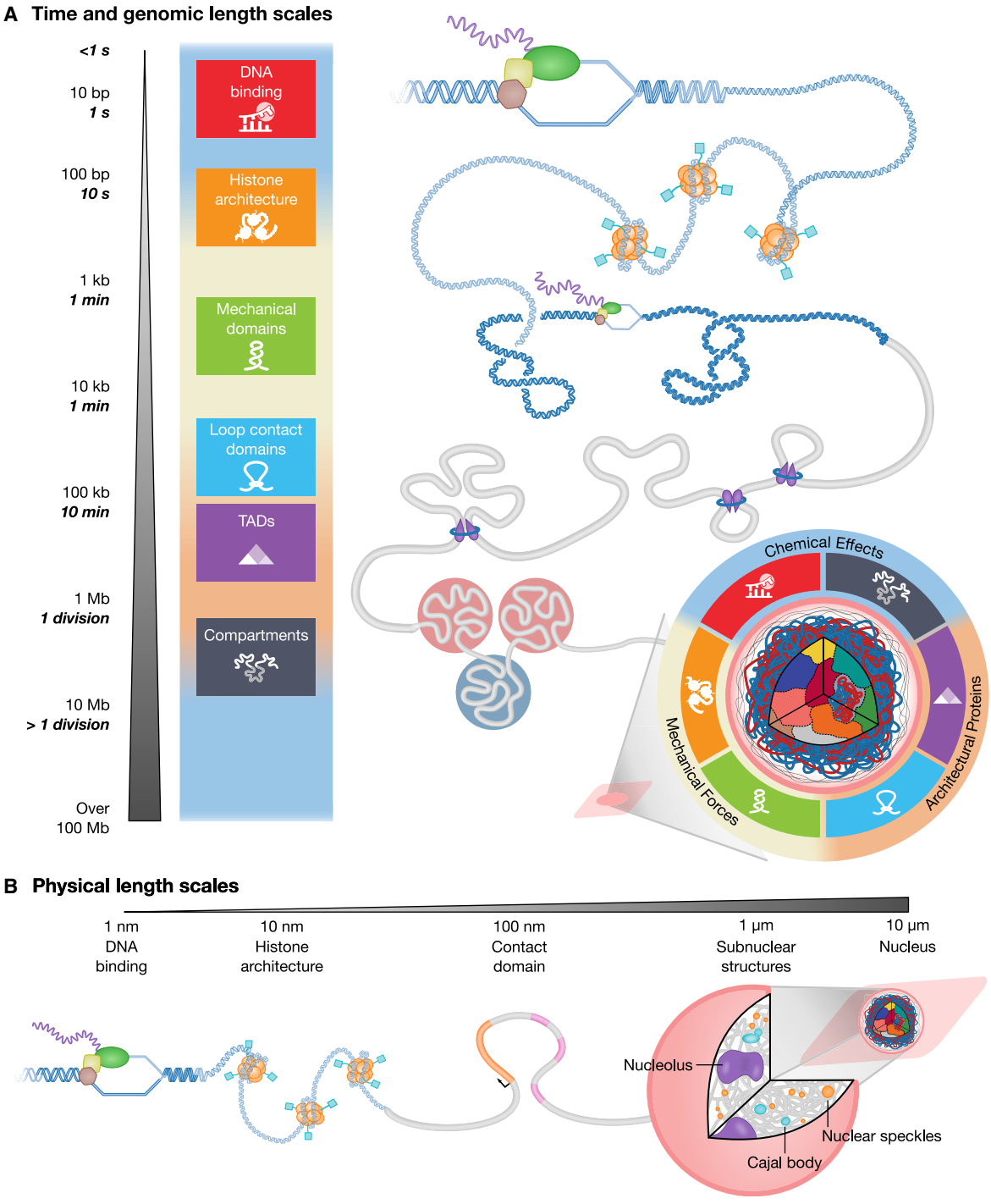

Figure 1. Scales and Modes of Chromatin Structure and Organization

The organization of cellular chromosomes extends over multiple overlapping genomic and physical length, and timescales.

(A) Time and genomic length scales: Numerous forms of organization have been identified ranging from small-scale patterns of histone positioning at the 1-kb-length scale, mechanical domains extending into the 10-kb scale, contact domains on the scale of 100s of kb and whole regions of chromosomes on the 1-Mb scale. Dynamic processes affecting each length scale occur on timescales that typically scale with the relevant length scale. A combination of chemical effects, mechanical forces, and architectural proteins contributes to the formation of each organizational mode. The distinct but overlapping modes help to link DNA base-pair-level activities, such as transcription and DNA binding, to large-scale structures such as DNA loops and chromosomal compartments. These links provide important mechanisms for the dynamic interactions of chromosomal structures and functions.

(B) Physical length scales: Multiple layers of organization also occur at different physical length scales spanning 5 orders of magnitude. At the largest scale, the nucleus is organized into chromosome territories and subnuclear structures like nucleoli. At the smallest scale of 1–5 nm, DNA-protein interactions are relevant (Dekker and Mirny, 2016).

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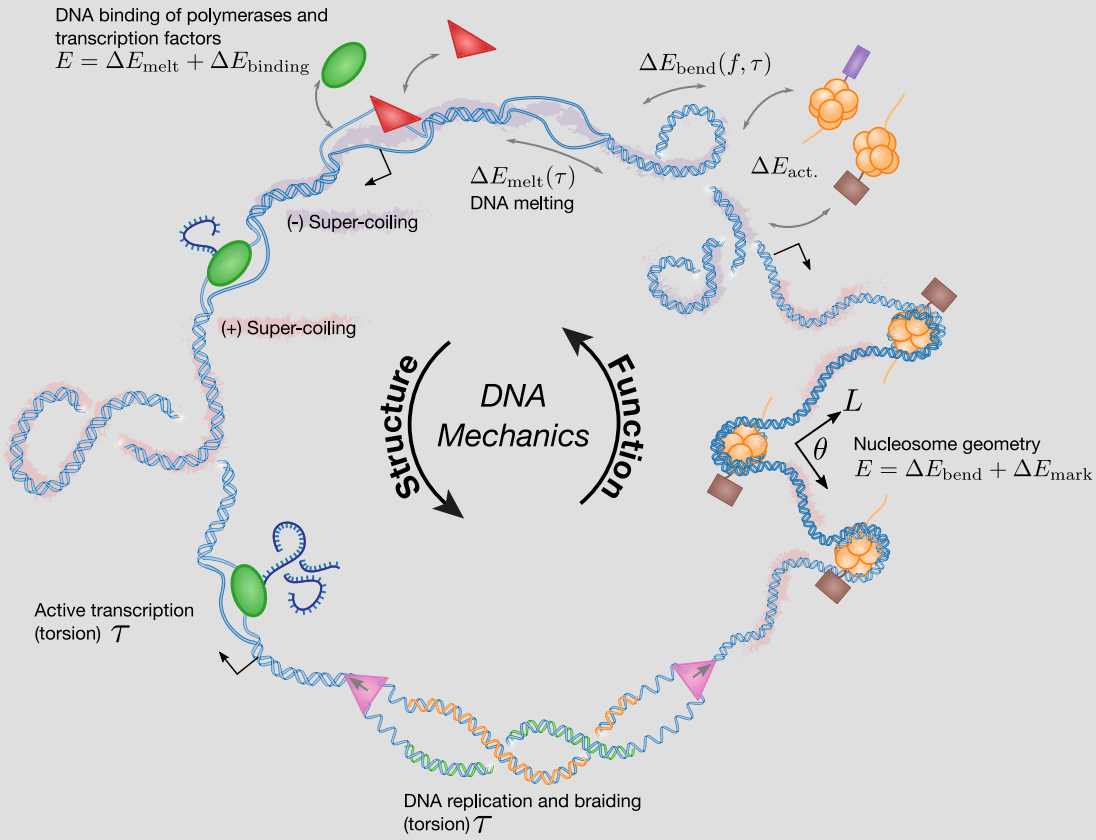
Box 1. Mechanical Epigenetics



Many DNA-based processes such as transcription, replication, and histone dynamics both control and are controlled by the mechanical state of DNA. We refer to this important layer of regulation and feedback as mechanical epigenetics.

To understand the role of mechanical epigenetics in gene regulation, it is important to highlight the physical properties of chromatin. DNA is composed of individual strands of covalently bonded nucleotides, which are then non-covalently paired with complementary strands (of order k_bT, see Glossary) to from the double helix. This relatively weak bonding makes local melting of the DNA possible through molecular interactions and use of ATP and NTP (~20 k_bT) which occur on the same energy scale (Marko, 2018). The helical nature of naked DNA introduces both substantial topological and mechanical properties into the polymeric behavior of DNA with bending and twisting persistence on the nm scale (Marko, 2018). To form chromatin, DNA must be physically wrapped around nucleosomes, which requires the chemical energy of nucleosome-DNA attraction to overcome mechanical energy associated with wrapping (Fierz and Poirier, 2019). In yeast and human cells, transcriptionally induced supercoiling demarks bounds of gene activity (Achar et al., 2020; Baranello et al., 2018; Corless et al., 2014; Naughton et al., 2013) and has been implicated in important structural (Le and Laub, 2016) and functional (El Houdaigui et al., 2019; Kim et al., 2019a) properties of bacterial transcription. Biophysical models of gene expression and DNA replication predict changes in chromatin structure that feed back into changes in transcriptional activity (Sevier, 2020; Sevier and Levine, 2018). The propagation of local mechanical forces and torques applied to DNA have the ability to influence DNA-bound processes at long distances (>10 kb). Thus, mechanical epigenetics create a hidden, dynamic interaction across many processes and scales. For instance, RNAPII is capable of exerting forces of up to 50 pN and torques of over 10 pN–nm, sufficiently forceful to influence histone binding and induce folded, buckled regions of chromatin (Fierz and Poirier, 2019; Le et al., 2019; Ma and Wang, 2016; Teves and Henikoff, 2014). Recent experiments have started to reveal a rich relationship between transcription and nucleosome positioning and the underlying mechanical state of DNA (Hsieh et al., 2020; Naughton et al., 2013). These phenomena collectively link dynamics on the timescale of DNA twisting (sub-second) to dynamics on the timescale of DNA bending (seconds to minutes) (Marko, 2018), which can be further integrated by histone and large-scale chromatin conformational changes (hours) (Fierz and Poirier, 2019). Thus, mechanical effects provide an important avenue for information storage between DNA-bound processes and chromatin organization, leading to a fundamental connection between chromosome function and structure.

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Box 1. *Continued*

As yet, few genomic analyses incorporate supercoiling assays into integrative analyses due to the relatively low signal of the assay (Corless et al., 2014; Teves and Henikoff, 2014). As improved supercoiling probes are developed, supercoiling structures may explain the connectivity between transcription and small-scale structures that predict differences in gene expression. Connecting single-cell tracking of transcriptional activity and small-scale structural features will provide better resolution of the casual link between these structures and gene expression.

interactions (1–5 nm) (Dekker and Mirny, 2016), cellular functions spanning long timescales are dynamically mediated by overlapping scales of genome organization. For mammals, the total chromosomal length exceeds 1 billion nucleotides. If arranged end-to-end and stretched-out length wise, the combined length of human chromosomes can span two meters (Alberts et al., 2002; Milo and Phillips, 2015). Each macroscopically long chromosome is packaged into a nucleus with a 10-μm diameter, requiring a compaction of 5 orders of magnitude (Figure 1B) (Milo and Phillips, 2015). Encoded DNA sequences determine DNA base pairing, transcription factor recognition and binding, and local DNA structures. Biochemical modification of DNA via methylation and other functional groups impacts protein binding and recognition in ways that can be propagated over many cell cycles (Liu et al., 2016; Seisenberger et al., 2013).

Beyond the linear sequence, the first major building block of chromatin organization is the nucleosome. Nucleosomes are 10-nm-sized protein complexes composed of eight histones wrapped ~1.6 times by a ~146-bp length of DNA (Fierz and Poirier, 2019; Luger et al., 1997). The position and geometry of nucleosomes within a chromosome represents a primary layer for encoding spatial and structural information in the genome that forms the basis of nucleosome-mediated epigenetics (Lai and Pugh, 2017). Two factors control the occupancy and positioning of histones: the affinity of the histone to DNA and the energy required to bend the DNA around the histone (Zuiddam et al., 2017). Histone modifications, such as acetylation and methylation on specific histone residues, alter both the physicochemical properties of the histone and the affinity of histone-DNA binding. While early *in vitro* studies of chromosomes at high salt concentrations revealed periodic organization of many histones into a 30-nm fiber, recent experiments reveal an interphase nucleus that is decorated with heterogeneous fibers of chromatin composed of clusters of nucleosomes (discussed further alongside imaging methods) (Maeshima et al., 2019; Ou et al., 2017).

On the length scale of tens of kb, multiple patterns of increased chromosomal contact exist between and within coding and noncoding regions of the genome. At this scale, many genomic elements support important cell-type-specific behaviors (Beagan et al., 2020; Kragesteen et al., 2018). These include changes involving coding elements, such as increased physical interactions between promoters and enhancers, as well as additional influences from long noncoding RNA (lncRNA), insulators and silencers. Consequently, methods of assaying proximity by identifying DNA contacts have driven recent efforts to understand gene regulation in eukaryotes (Figure 2) (Beagan et al., 2020; Hsieh et al., 2020; Lu et al., 2020; Oudelaar et al., 2020).

Cells manipulate the local and global mechanical state of chromatin using enzymes and energy to induce forces and torques on chromatin. Mechanical forces are transmitted to the chemical state of chromatin by altering the distribution of DNA and nucleosome bound molecules. Recent sequencing-based techniques have revealed contact domains (see Glossary) of chromatin spanning 5-kb genomic distances that are highly correlated with active transcription (Hsieh et al., 2020; Krietenstein et al., 2020; Rowley et al., 2017). This length scale coincides with observed regions of chromatin over and under twisting (Baranello et al., 2018). This largely hidden phenomena, referred to as DNA supercoiling (see Glossary), can mechanically influence higher-order histone structures across many kilobases (Bancaud et al., 2006; Le et al., 2019) and dynamically alters and is altered by DNA-based processes such as transcription and replication (Figure 1; Box 1) (Ma and Wang, 2016). The role of mechanical effects such as supercoiling have long been linked to transcription (Ma and Wang, 2016) and chromatin organization (Baranello et al., 2018; Corless and Gilbert, 2016). Recent studies have implicated transcriptional mechanics in the structure (Le and Laub, 2016; Le et al., 2019; Marinov et al., 2020) and function (El Houdaigui et al., 2019; Kim et al., 2019a; Yeung et al., 2017) of lower organisms and mammalian systems (Desai et al., 2020; Leidescher et al., 2020).

At the 100-kb length scale, larger contact domains are referred to as topologically associated domains (TADs, see Glossary) (Rowley et al., 2017). Contact domains are often marked by point-like contacts between domain boundaries ("corner dots" in Hi-C maps). In mammals, domain boundaries often display high association with cohesin, the structural maintenance of chromosomes (SMCs) protein, which forms loops by binding two strands of chromatin. These contact domains are dubbed loop domains (Brandão et al., 2019; Fudenberg et al., 2016; Rowley et al., 2017; Sanborn et al., 2015). Enrichment of intra-domain contacts seen inside TADs may explain how the effects of *cis*-regulatory elements like enhancers are restricted to specific contact domains (Schoenfelder and Fraser, 2019; Symmons et al., 2014). Beyond providing boundaries and structure, the functional importance of TADs on gene regulation remains unclear (Beagan and Phillips-Cremins, 2020).

At the Mb length scale, areas of active and inactive chromatin spatially segregate into micron-sized regions called compartments (see Glossary) (Mirny et al., 2019). Early nomenclature dubbed regions of chromatin as A or B compartments if they contained accessible or inaccessible chromatin, respectively (Rowley et al., 2017). Early genome-wide contact maps were only able to detect contacts between large Mb regions, resulting in large A/B compartments (Rao et al., 2014). However, higher resolution techniques identify finer A/B-like compartments, called compartmental domains. Compartmental domains more closely correlate with transcriptionally active and inactive regions than the originally characterized larger A/B compartments

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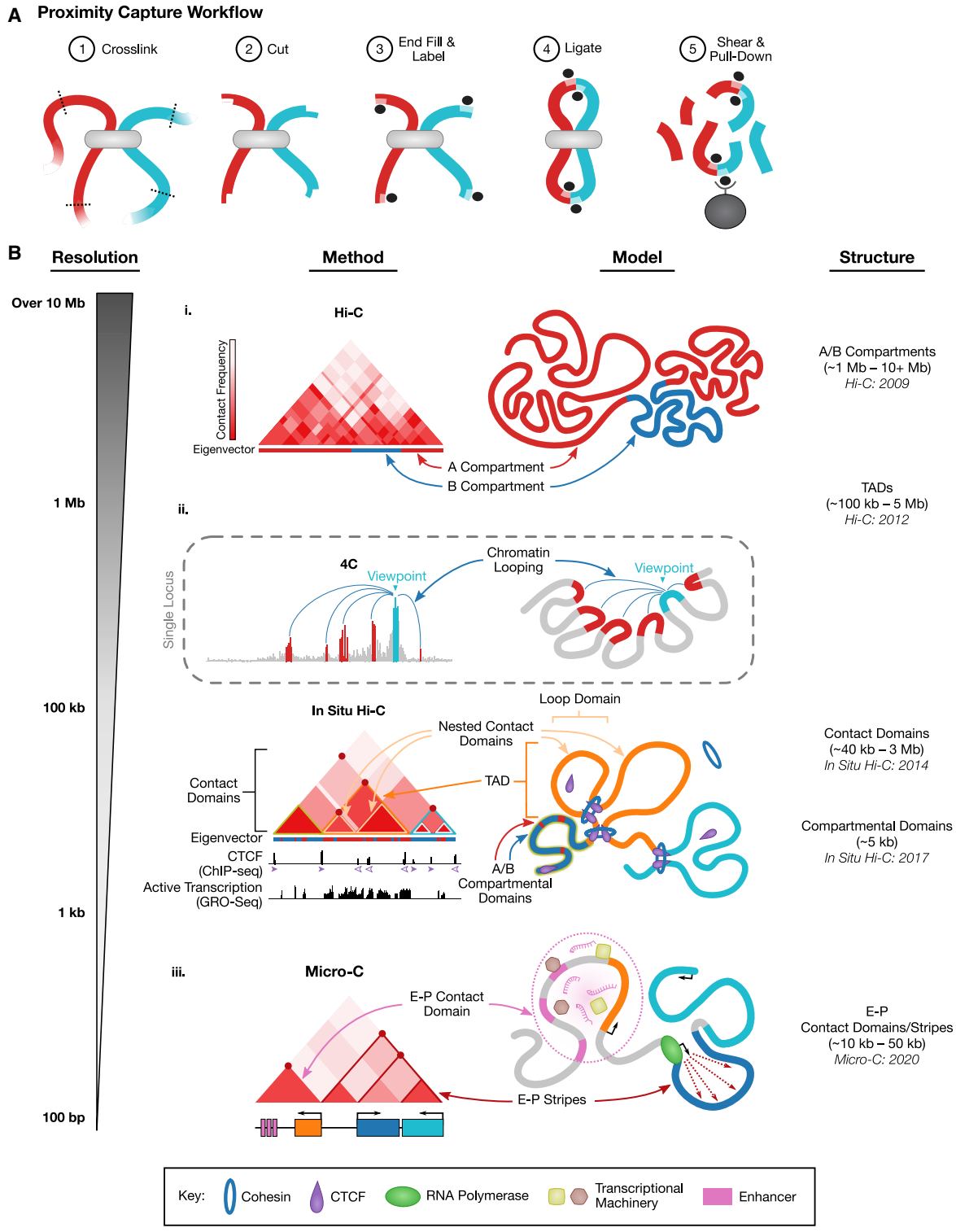

Figure 2. Sequencing-Based Approaches to Probing Chromatin Structure and Organization

The increasing resolution of 3C-derived sequencing methods have revealed different principles of chromatin organization at increasingly fine length scales. (A) Proximity capture workflow: All 3C-derived methods use a proximity capture workflow to produce a library suitable for next generation sequencing by crosslinking chromatin to nearby neighbors, digesting it into fragments, followed by label, ligation, and pull-down steps to isolate loci of interest. (B) (Bi) Hi-C: Early Hi-C protocols ligated fragments under dilute conditions and with 6-bp cutters, limiting the structures observed to large triangular Mb regions, called TADs. (Bii) 4C and *in situ* Hi-C: 4C (top) detects long-range chromatin contacts, often attributed to looping, with a single viewpoint. *In situ* Hi-C (bottom)

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(Hsieh et al., 2020). Compartmental domains of both types (e.g., A/B) exist within single loop domains and outside of loop domains (Rao et al., 2014). Compartments also display long-range attraction, microphase separation (see Glossary), and nested hierarchies (Mirny et al., 2019; Rowley et al., 2017; Rowley and Corces, 2018).

Given the diverse scales and temporally distinct modes of gene regulation, understanding how chromatin's dynamic structure determines gene regulation and cellular identity remains a grand challenge. Recent advances in technologies for studying chromatin structure are illuminating the principles and scales of organization with increasing resolution. While real-time observations shed invaluable light on the dynamic nature of chromatin, static pictures can shed light on entire regions of chromatin at multiple length scales. The emergent structures revealed in static observations provide insights into the size, scale, control, and components of chromatin organization. However, perturbative bottom-up methods in conjugation with mechanistic theoretical connections must be made to connect dynamic and static perspectives. Thus, a complete picture of chromatin as a dynamical system involves the blending of both dynamic and static observations from both top-down and bottom-up methodologies (see Glossary).

SEQUENCING-BASED METHODS UNCOVER MULTIPLE LENGTHS SCALES OF GENOME ORGANIZATION

One major class of methods to map 3D genome organization relies on sequencing-based techniques. Although the 3D genome is structurally dynamic, snapshots of physical contacts between chromatin in 3D space can reveal important aspects of chromatin organization. Contacts are commonly detected with chromosome conformation capture (3C)-based methods that use proximity ligation of physically close DNA fragments to measure contact frequencies between loci of interest (Figure 2A) (Dekker et al., 2002). Though these methods only reveal static configurations of chromatin, they have proven invaluable in revealing the diversity of structures present. Various 3C-based techniques (e.g., 3C/4C/5C/Hi-C) differ in the number of genomic loci from which contacts can be simultaneously detected (Dostie et al., 2006; Lieberman-Aiden et al., 2009; Zhao et al., 2006). For example, 4C probes all contact partners for a locus of interest (i.e., one to all), whereas Hi-C detects all contacts genome wide (i.e., all to all). Other techniques like HiChIP and ChIA-PET enrich for contacts bearing specific DNA-protein interactions (Li et al., 2014; Mumbach et al., 2016). All 3C-based methods require the generation and sequencing of DNA fragments for proximity ligation. Therefore, sequencing depth and fragment length determine the smallest detectable units of structural organization (i.e., maximum level of resolution) obtained (Denker and de Laat, 2016).

Hi-C methods generate population-averaged genome-wide contact frequency maps (Figure 2) (Dixon et al., 2012; Lieberman-Aiden et al., 2009). The sequencing depth of an early Hi-C study enabled identification of 1-Mb regions with increased contact frequencies (Lieberman-Aiden et al., 2009). With 1-Mb resolution, large 1 Mb to >10 Mb regions that interact over long length scales (creating a characteristic checkerboard pattern in contact frequency heatmaps) were identified as corresponding to transcriptionally active and inactive A and B compartments, respectively (Figure 2Bi) (Lieberman-Aiden et al., 2009). At 40-kb resolution, triangle patterns ranging from 100 kb to 5 Mb emerge on contact heatmaps, indicating increased contact frequencies between loci within those regions (Dixon et al., 2012). Due to their proximity and putative physical association that is facilitated by chromatin folding, these domains were named TADs (Dixon et al., 2012; Nora et al., 2012). Enrichment of CCCTC-binding factor (CTCF) often marks the borders of these TADs, which display some conservation across cell types (Beagan and Phillips-Cremins, 2020; Bonev et al., 2017).

Original 3C methods used 6-bp restriction enzyme cutters to produce DNA fragments and performed proximity ligation under dilute conditions. By using a 4-bp cutter to generate shorter DNA fragments and by performing ligation in intact nuclei (*in situ* proximity ligation, see Glossary) to reduce noise, the maximum resolution of Hi-C drastically increases to 1 kb (Rao et al., 2014). *In situ* Hi-C reveals finer domain boundaries (Figure 2Bii), indicating that many TADs are hierarchically organized into smaller 40 kb to 3 Mb nested contact domains (sometimes called subTADs) (Phillips-Cremins et al., 2013; Rao et al., 2014). Many of these smaller contact domains display specificity that is linked to developmentally regulated genes and cell types (Phillips-Cremins et al., 2013). Furthermore, a number of these finer domains are marked by strong corner dots, thought to be caused by chromatin loops (Beagan and Phillips-Cremins, 2020).

Recently, through an improved fragmentation protocol, the resolution of contact maps has increased 5-fold (Hsieh et al., 2016). Previously used restriction enzymes generate heterogeneous distributions of fragments and are limited to a maximum resolution of 1 kb, even in the absence of limitations such as sequencing depth and cell number (Hsieh et al., 2016, 2020). In contrast, Micro-C uses micrococcal nuclease (MNase) to fragment DNA into evenly spaced mononucleosomes, achieving 200-bp resolution with only tens of thousands of cells while still capturing structures over 1 Mb (Figure 2Biii). Enhanced mapping via Micro-C identified previously undetected features, such as 10 kb to 50 kb nested contact map stripes (Hsieh et al., 2020). Many of these stripes are punctuated by dots when they intersect and connect enhancer-promoter (E-P) sites or promoter-promoter (P-P) sites in so-called E-P domains (Hsieh et al., 2020). Furthermore, finer-scale domain boundaries (<1 kb) encompassing only 1–2 genes in E-P domains were found exclusively in active compartment A regions and were marked by different biochemical and functional features (e.g., transcription factor-binding sites, embryonic-stem-cell-specific domains, etc.) (Hsieh et al., 2020). If these newly detected Micro-C dots correspond to transient loops and how these domains relate to larger stable loop domains such as TADs remains unclear

improved the resolution of Hi-C, leading to the detection of finer compartmental domains (red and blue eigenvector regions), nested contact domains (light orange and blue triangles), and loop domains (dark red dot) occurring inside larger TADs (green, blue, and orange triangles). Mammalian loop domains are often flanked by convergent CTCF-binding sites (purple arrows). (Biii) Micro-C: At a maximum resolution of ~200 bp, Micro-C detects E-P contact domains and E-P stripes that connect E-P and P-P sites, enclosing as little as 1 to 2 genes.

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(Hansen et al., 2017; Krietenstein et al., 2020). These observations also highlight how increasingly high-resolution contact maps can help discover previously undetected, dynamic, genome-wide interactions (e.g., E-P domains, Micro-C-specific dots).

Although 3C-based methods are widely used to probe 3D genome organization, the proximity ligation step required in 3C-derivatives may miss important interactions that do not occur close enough for molecules to proximity ligate to one another (Beagrie et al., 2017; Quinodoz et al., 2018, 2020). Thus, non-proximity methods may enable mapping of structures beyond this limit. Genome architecture mapping (GAM) isolates thin cryosections of nuclei, then amplifies and sequences the DNA in each nuclear slice to identify interacting genomic loci (Beagrie et al., 2017, 2020). Another sequencing technique that avoids proximity ligation relies on split-pool recognition of interactions by tag extension (SPRITE). Specifically, SPRITE crosslinks RNA and DNA within the nucleus together, fragments it, then uses a ''split-pool tagging'' process to uniquely barcode each crosslinked fragment. This split-pool barcoding approach allows both RNA and DNA to be directly read, enabling the simultaneous identification of DNA-DNA, RNA-DNA, and RNA-RNA interactions (Quinodoz et al., 2018, 2020). Average 3D chromatin structures are similarly recapitulated by Hi-C, GAM, and SPRITE methods (Fiorillo et al., 2020). However, recent improvements to SPRITE have greatly increased the detection efficiency of low-abundance RNAs (e.g., noncoding RNAs and nascent mRNAs), revealing the importance of RNA in guiding other RNAs and proteins to specific subnuclear structures and genomic loci (Quinodoz et al., 2020). These observations highlight the importance of orthogonal approaches to studying 3D genome organization.

While high-resolution contact maps have enabled the identification of 3D chromatin structures at extremely fine scales, many 3C-based techniques average sparse contacts over a single temporal snapshot of millions of cells (Beagan and Phillips-Cremins, 2020). Given that 3D genome organization is highly dynamic, it is unsurprising that single-cell Hi-C data and computational methods to compute polymer models consistent with this data reveal that inter-domain interactions occur more frequently and with larger heterogeneity than suggested by population maps (Flyamer et al., 2017; Stevens et al., 2017; Tan et al., 2018). Thus, the domains identified from population-averaged contact maps, such as compartmental and contact domains, putatively represent the most coherent or probable structures of an entire structural ensemble. Combining sparse single-cell Hi-C data with imaging techniques confirm that loop domains do not form in all cells and are heterogeneously located (Flyamer et al., 2017; Stevens et al., 2017). Studies like these demonstrate how combined sequencing and imaging-based single-cell methods may improve our understanding of dynamic nuclear architecture at the single-cell level.

SINGLE-CELL IMAGING METHODS REVEAL EXTENSIVE HETEROGENEITY AND DYNAMIC BEHAVIOR AT THE VARIOUS LENGTH SCALES OF GENOMIC ARCHITECTURE

Imaging methods constitute a complementary category of approaches to mapping nuclear architecture. Imaging methods provide static and dynamic single-cell examinations of nuclear architecture beyond averaged chromosomal points of contact (Figures 3 and 4). Chromatin imaging methods can be broadly classified into three categories: fluorescence *in situ* hybridization (FISH) methods (see Glossary), which collect the fine-scale multi-probe spatial localization of static chromatin conformations; fluorescent protein methods, which can track dynamic live-cell chromatin motion; and non-fluorescent methods, which offer extremely detailed insight into static configurations of chromatin.

Fluorescent protein tags and DNA or RNA FISH probes can be imaged via diffraction-limited fluorescence microscopy (Lakadamyali and Cosma, 2020). FISH relies on probes hybridizing to their targets in the nucleus and thus requires fixed cells, as the fixation process ensures that the probes can permeate to the nucleus and that chromatin structure remains intact during the high-temperature denaturing step. Initial FISH studies revealed large-scale organization such as lamina-associated domains (LADs), regions of the genome that preferentially interact with the nuclear lamina and are often transcriptionally silenced (Guelen et al., 2008). Initial FISH-based methods used a probe library directly conjugated to reporter fluorophores, involving an expensive library construction step and limiting multiplexing without a harsh washout step to the number of distinguishable colors (Joyce et al., 2012; Raj et al., 2008). Oligopaint and its descendent methods circumvent expensive library creation by constructing and simultaneously incubating an entire library of identically labeled DNA probes (Beliveau et al., 2012). By labeling Mb regions of chromosomes with FISH probes, chromosome territories were directly imaged with Oligopaint in fixed cells (Beliveau et al., 2014, 2015). By separating the DNA-binding functionality from the imaging functionality, derivative methods use secondary fluorescent probes that hybridize to DNA target probes and perform strand displacement at room temperature, allowing successive regions as small as 10 kb to be distinguished with microscopy without harsh wash conditions (Nir et al., 2018). When combined with super-resolution microscopy (see Glossary), this class of methods, including both OligoSTORM and optical reconstruction of chromatin architecture (ORCA), can be used to determine the 3D spatial location of sequential chromosomal segments as short as several kb within the nucleus (Figure 3A) (Bintu et al., 2018; Mateo et al., 2019; Nir et al., 2018).

By using the median spatial distance between pairs of genomic loci to generate a Hi-C like contact map, structures that look like Hi-C contact domains (e.g., triangles) appear (Bintu et al., 2018). Single-cell imaging indicates that cohesin depletion results in variable domain boundaries, while leaving the Mb-sized A/B compartments relatively unaffected. When averaged over the entire population, this randomization of domain boundaries is responsible for the near-complete reduction in TADs observed in population-level Hi-C data (Bintu et al., 2018). Here, the dynamic motion of contact domain boundaries is inferred from the multi-cell ensemble, instead of being directly measured.

In contrast to the FISH-derived methods, protein-based methods capture nuclear real-time dynamics in living cells (Gu et al., 2018; Shaban et al., 2018). Protein fluorophores offer low quantum yield compared with the small-molecule dyes used in

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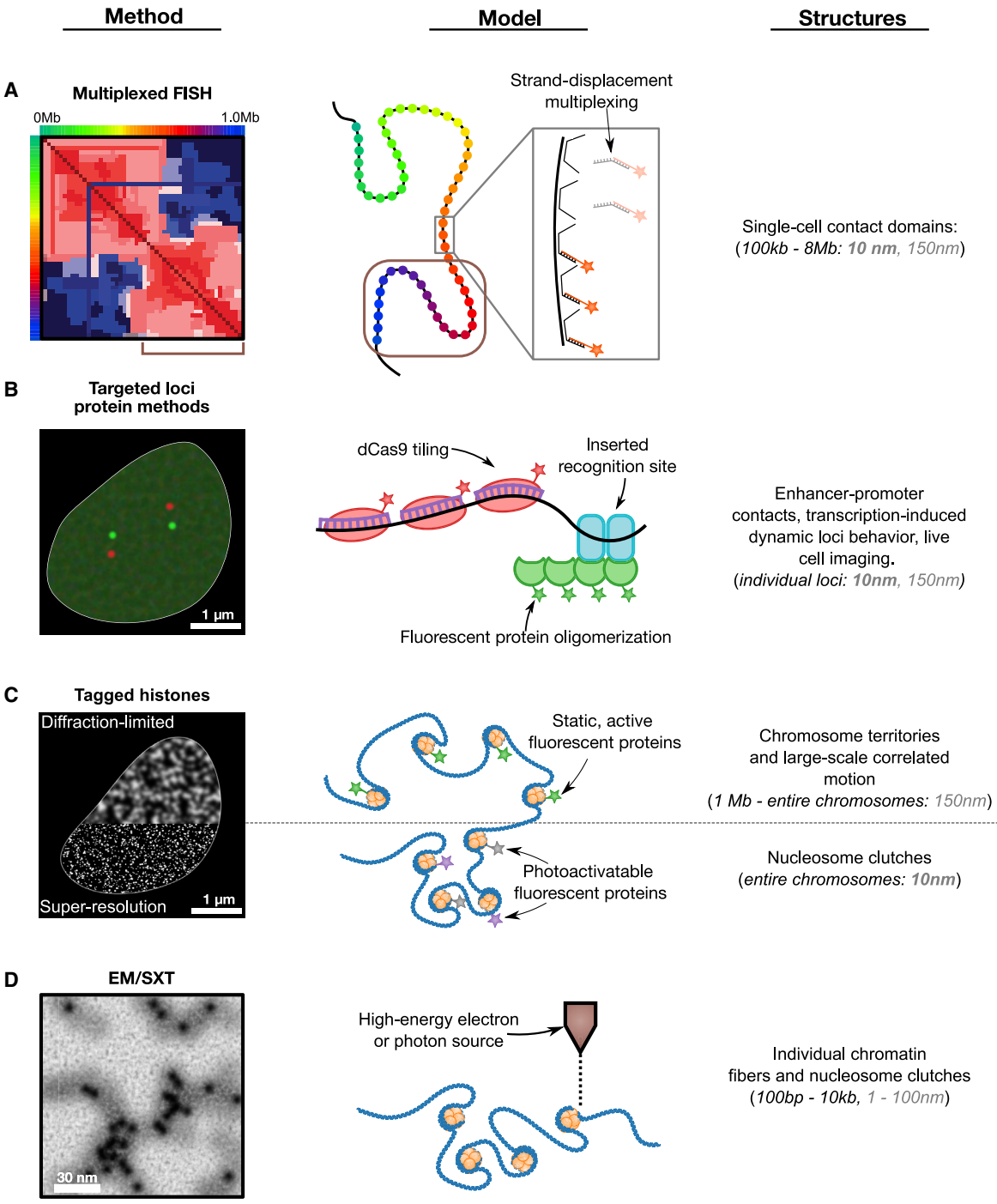

Figure 3. Imaging-Based Approaches to Probing Chromatin Structure and Organization

The heterogeneity of nuclear architecture at the single-cell level is revealed by various imaging methods. The relevant chromatin structures that each method can observe is determined by multiplexing ability and maximum imaging resolution (shown here both for super-resolution and diffraction-limited cases).

(A) Multiplexed FISH: By separating fluorescent readout and target binding into separate probes as in OligoSTORM and ORCA, successive genetic loci across megabase regions can be visualized (middle). Localizations can be summarized by median distance between loci pairs, giving a single-cell contact map matrix (left). Averaging single-cell contact map matrices from many cells reveals ensemble behavior similar to that observed with Hi-C.

(B) Targeted-loci protein methods: Target loci in live cells can be imaged via protein fusions of DNA-binding domains and fluorescent proteins. Both native loci and inserted recognition sites can be visualized. Multiplexing is limited by fluorescent protein color, with only around four simultaneous probes remaining distinguishable.

(C) Tagged histones: Histones tagged with fluorescent proteins can be visualized across the entire nucleus. In addition to imaging motion of large-scale chromatin structures, super-resolution allows identification of heterogeneous nucleosome clutches.

(D) EM/SXT: non-fluorescent methods including electron microscopy and soft X-ray tomography allow for maximum resolution approaching one nanometer, uncovering heterogeneities at the smallest relevant scale.

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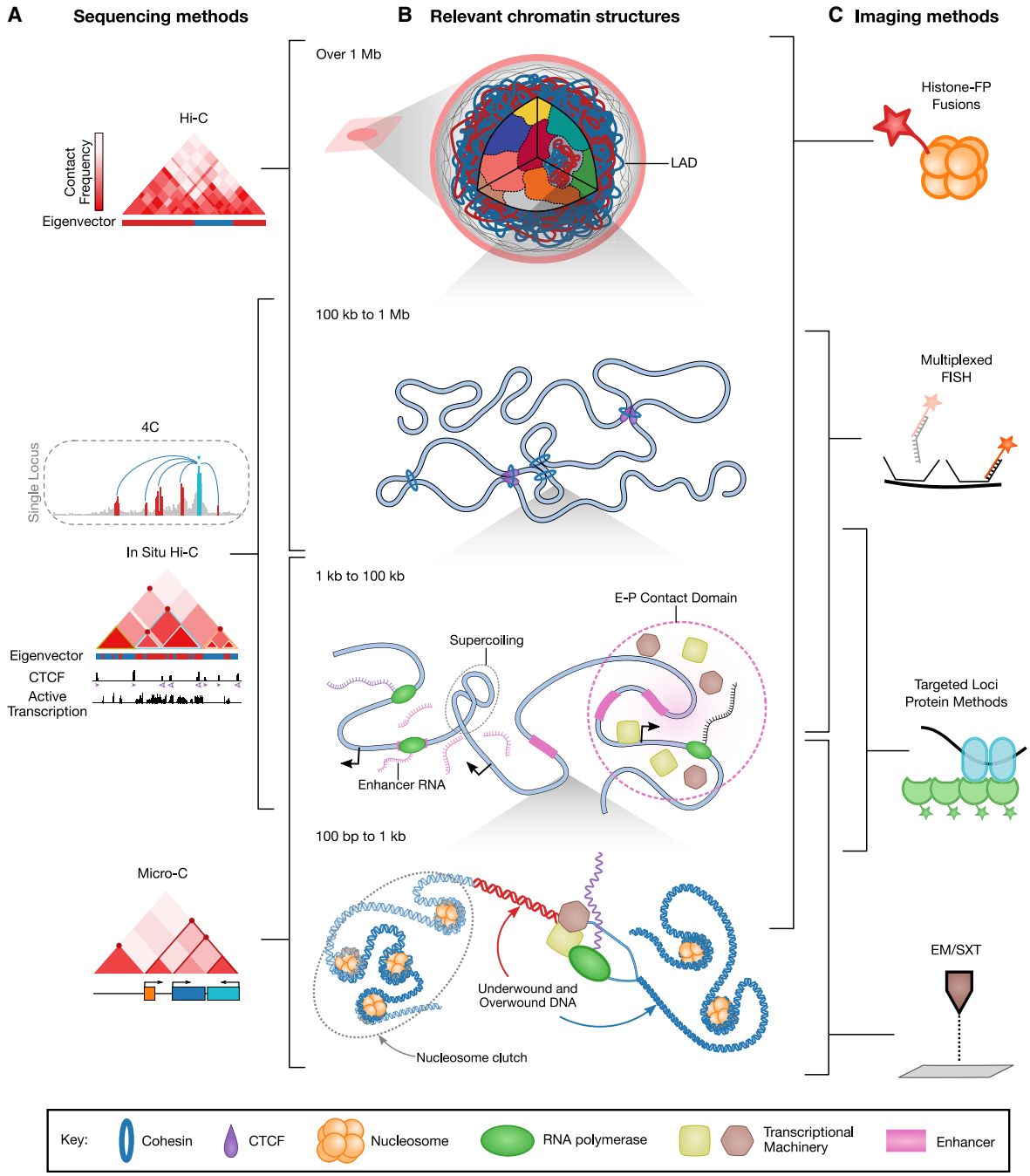

Figure 4. Sequencing- and Imaging-Based Approaches: An Integrated View to Understand Dynamic Chromatin Structures and Organization

(A–C) Dynamic chromatin architecture at various relevant length scales (B, center) is best understood via complementary information revealed by sequencing (A) and imaging methods (C).

(A) Sequencing methods: 3C-derived methods give population-level view of chromatin architecture. Initial Hi-C methods revealed large-scale compartments, LADs, and loop domains, whereas modern techniques including Micro-C reveal fine structures at the 200-bp level and can still probe large-scale structures.

(B) Relevant chromatin structures: Chromatin structures are nested and interlinked on length scales spanning over five orders of magnitude. Over 1 Mb: Chromatin segregates into chromosome territories, LADs, and Mb-sized A/B compartments. 100 kb to 1 Mb: Long-range looping interactions and large TADs, identified as triangles in Hi-C data, were first identified at this scale. 1 to 100 kb: Smaller nested contact domains exist at this scale. Dynamic processes such as transcription, enhancer-promoter interactions, and phase condensates reshape these contact domains. 100 bp to 1 kb: At the length scale of nucleosomes, chromatin is highly heterogeneous and is shaped by physical processes with energies not exceeding a few *kT*.

(C) Imaging methods: Microscopy techniques allow visualization of chromatin structures in single cells, revealing heterogeneity both in the boundaries of large contact domains and at the length scale of individual nucleosomes.

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FISH methods (Giepmans et al., 2006). A variety of methods exist to recruit multiple fluorophores to targets to improve detection (Figure 3B) (Lakadamyali and Cosma, 2020). In tiled dCas9-, TetR-TetO, and TALE-based systems (see Glossary), multiple proteins are recruited to adjacent locations on the genome (Chen et al., 2013; Miyanari et al., 2013; Straight et al., 1996; Tasan et al., 2018; Zhu and Cheng, 2020). In contrast, methods like ANCHOR recruit fluorophores by oligomerizing multiple fluorescent proteins to a single-protein-bound DNA site, allowing targeting of a single non-repetitive genetic locus (Germier et al., 2017). These methods allow for live measurement of the location and mobility of genomic elements (e.g., promoters and their *cis*-regulatory elements) for seconds to minutes. Dynamics at this scale display multiple modes of behavior from generic confinement (Khanna et al., 2019) to decreased mobility of a gene's promoter region with increasing activity (Germier et al., 2017). However, the mobility of *cis* enhancers increases as transcription rate increases, consistent with a model where active transcription-linked processes increase the frequency of interactions with regulatory elements (Gu et al., 2018). The dynamic behavior of the entire nucleus can also be studied through these protein-based methods. One study used fluorescently tagged histone proteins to characterize whole-nucleus chromatin motion and found that chromatin motion was correlated over length scales equal to nearly a tenth of the nuclear diameter (Figure 3C) (Shaban et al., 2018). Furthermore, inhibiting RNA polymerase II (RNAPII) elongation resulted in a decrease of both correlated chromatin motion and diffusion, suggesting that transcription is an important regulator of coordinated chromatin motion (Shaban et al., 2018, 2020). These protein-based imaging techniques will be invaluable in uncovering transcription dynamics and other processes that cannot be interrogated in fixed cells.

At the smallest scale, nuclear architecture can be captured via fluorescent and non-fluorescent techniques. Histone-fusion proteins allow for super-resolution live-cell imaging of the entire nucleus at 20-nm resolution (Figure 3C) (Ricci et al., 2015). Fluorescent, super-resolution, live-cell studies demonstrated that nucleosomes were arranged in visually distinct grouping called nucleosome clutches that vary in density along chromatin fibers (Lakadamyali and Cosma, 2020; Ricci et al., 2015). Both cell type and local transcriptional activity affected the density of these nucleosome clutches (Ricci et al., 2015).

Sub-nucleosome resolution up to 1 nm can be achieved via non-fluorescent electron microscopy techniques such as ChromEMT or via soft X-ray tomography (Figure 3D) (Le Gros et al., 2016; Ou et al., 2017; Smith et al., 2014). In ChromEMT, diaminobenzidine polymerized onto chromatin is visualized by staining. ChromEMT images of human cell nuclei revealed that, in contrast to the original *in vitro* understanding of hierarchical 30-nm chromatin fibers, chromatin in interphase chromosomes is organized as distributed chromatin fibers of varying diameter. These observations confirm the heterogeneous distribution of nucleosomes observed as ''clutches'' in both imaging and sequencing technologies, in static and dynamic observations, and in single-cell and bulk structure analyses. Although non-fluorescent methods allow 1-nm resolution, these methods necessarily provide a static view of the nucleus. Recent advances in super-resolution microscopy extend live-cell imaging to the 1 nm level (Gwosch et al., 2020). Because processes affecting chromatin at this scale operate on the timescale of milliseconds to seconds (such as nucleosome binding and transcription elongation), live-imaging methods will be critical in understanding the finest scales of intricate nuclear dynamics.

ADVANCES IN ENGINEERING CHROMATIN AND NUCLEAR ARCHITECTURE

Simultaneously tracking all relevant phenomena impacting chromatin structure makes conventional top-down deconstruction of chromatin's structure-function relationship challenging. Alternatively, synthetic, bottom-up approaches for constructing synthetic models of chromatin may lead to invaluable insight into how structures emerge from functions. These insights may identify mechanisms that feedback into chromosome function by revealing the causal processes underlying chromatin dynamics through perturbative experiments.

ENGINEERING TRANSITIONS IN CHROMATIN STATE AND CELL FATE VIA SYNTHETIC TRANSCRIPTION FACTORS

Unique transcriptional and epigenetic profiles mark and maintain cellular identities (Spivakov and Fisher, 2007). Transitions in cell fate require chromatin remodeling which may be facilitated by transcription factors (Black et al., 2016; Ieda et al., 2010; Vierbuchen et al., 2010). However, understanding how small-scale (10 bp), high-frequency transcription factor dynamics induce stable cell-fate changes is poorly understood. In reprogramming and direct conversion, transcription factors drive changes in chromatin structure directly by binding to cognate sequences, recruiting transcriptional machinery, and inducing transcription (Di Giammartino et al., 2019; Stadhouders et al., 2018). Additionally, transcription factors drive indirect changes in chromatin structure via the activation of transcriptional networks, inducing expression of additional transcription factors and epigenetic regulators (Cahan et al., 2014). Consequently, efforts to drive cellular transitions have focused on transcription factor cocktails to induce specific cell fates. While directed differentiation via small molecules and exogenous factors represents the most common method of generating somatic cells types from iPSCs, overexpression of lineage-specifying transcription factors is increasingly employed to guide cells to specific identities (Flitsch et al., 2020; Nickolls et al., 2020; Wang et al., 2017). The development of synthetic transcription factors expands the ensemble of tools beyond native transcription factors to enable precise single-locus and multi-locus targeting of regulatory nodes. These efforts may reveal the mechanistic connections between properties of transcription factors, chromatin dynamics, and cell fates.

Synthetic transcription factors are composed of two primary domains: a DNA-binding domain and an activation domain (Figure 5A). Selection of the DNA-binding domain determines sequence specificity, allowing focused activation of a single locus or connection to broader transcriptional networks via native transcription factor-binding sites. CRISPR activator (CRISPRa) systems employ programmable gRNAs to target specific regions (Chavez et al., 2016). CRISPRa proteins are composed of catalytically inactive Cas9 (dCas9) fused to various activation domains (Figure 5A). Activation domains of CRISPRa include

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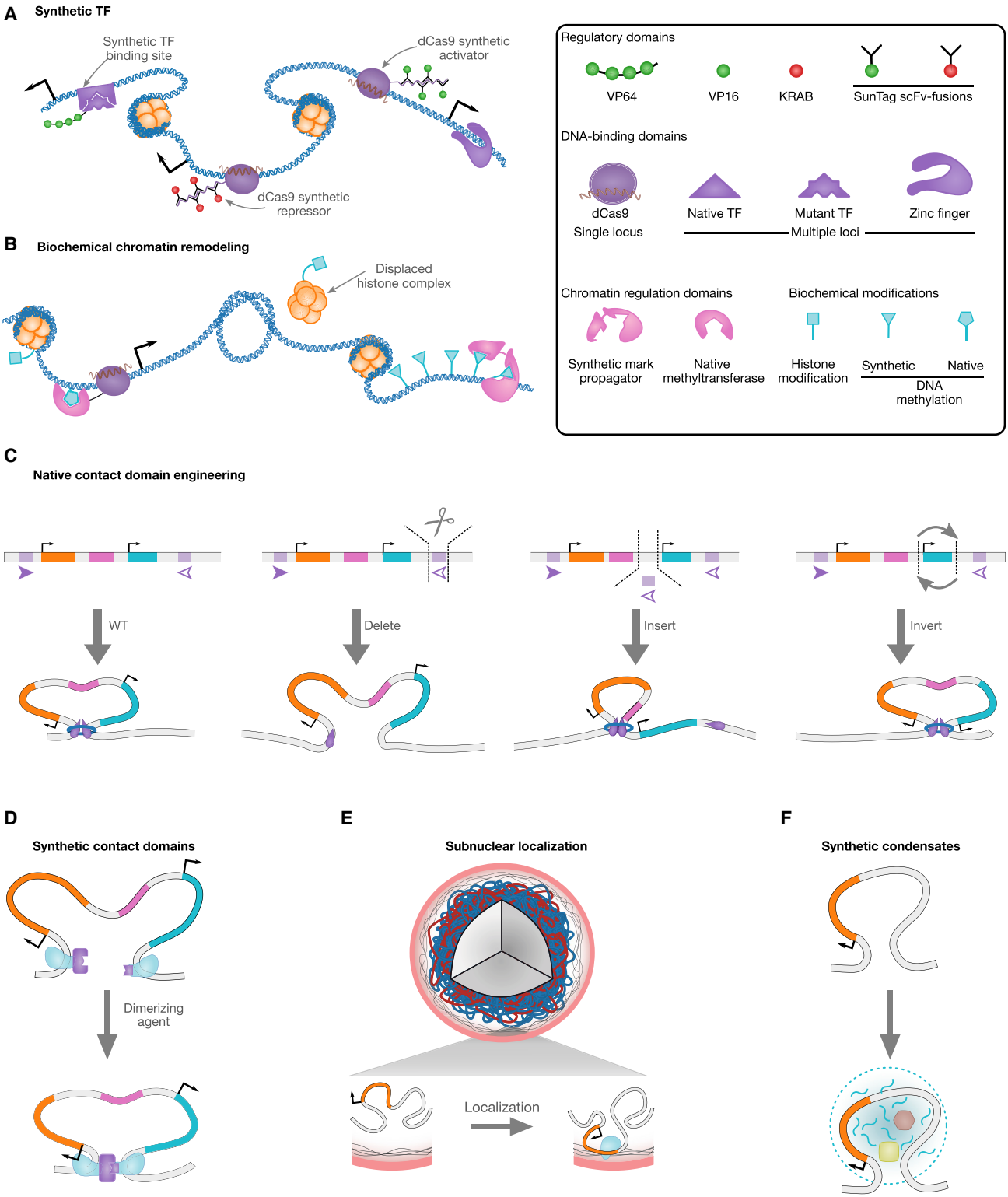

Figure 5. Engineering Chromatin

(A) By fusing various DNA-binding domains to known transcription regulators, synthetic transcription factors can selectively regulate native and synthetic gene circuits. These transcriptional regulators can be recruited to specific single- and multi-loci targets. Recruitment of regulatory domains can be enhanced via the SunTag system.

(legend continued on next page)

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variants of VP16 and p65 (e.g., VP64, VPR) as well as p300 that are either fused to dCas9 or recruited to dCas9 via protein-protein or protein-RNA interactions. Design of gRNAs of varying affinity provides a method for tuning CRISPRa-induced gene expression to more precisely control activation of various loci utilizing a single CRISPRa protein (Jost et al., 2020). Activation domains induce transcription by recruiting transcriptional machinery to transcription start sites and enhancers. Multimerization of activation domains via multi-valency domains enhances recruitment and gene activation (Tanenbaum et al., 2014). Off-target effects of CRISPRa systems appear to be minimal compared with off-target effects of CRISPR screening systems (Gilbert et al., 2014).

Synthetic transcription factors can replace overexpression of native transcription factors to induce reprogramming. Key pluripotency transcription factors such as Oct4 and Sox2 are overexpressed in order to reprogram fibroblasts into induced pluripotent stem cells (iPSCs). Instead of overexpressing these genes, targeted activation of *Oct4* and *Sox2* via CRISPRa successfully reprograms iPSCs (Balboa et al., 2015; Liu et al., 2018). Similarly, targeting CRISPRa to the myogenic transcription factor *Myod1* enables direct conversion of mouse embryonic fibroblasts (MEFs) to skeletal muscle (Black et al., 2016; Chakraborty et al., 2014). Activation of other key transcription factors with CRISPRa can convert MEFs into cardiomyocyte progenitor cells or neural cells (Black et al., 2016; Wang et al., 2020b). Together these data suggest that nucleated activation at key regulatory nodes is sufficient to induce the massive chromatin remodeling required for cellular reprogramming. Reprogramming remains a rare event within a population of cells. Insight into the mechanisms that limit reprogramming may provide opportunities for synthetic transcription factors to target additional regulatory nodes in the epigenome.

While CRISPRa techniques provide programmable site-specific activation, activity often requires multiple guides per locus which makes scaling to larger transcriptional networks challenging. Additionally, the large size of dCas9 (4.5 kb gene) limits adoption into viral vectors with smaller cargo limits (e.g., adeno-associated virus [AAV]). Single guide strategies with smaller Cas variants may enable improved flexibility of CRISPRa (Pausch et al., 2020; Zetsche et al., 2015). Alternatively, sequence specificity can also be programed through smaller native transcription factors (Kabadi et al., 2015). Beyond native transcription factors, directed evolution of native factors or artificial zinc fingers libraries has generated synthetic transcription factors that can replace reprogramming factors (Eguchi et al., 2016; Veerapandian et al., 2018). Improvement in synthetic transcription factors used for cellular reprogramming may benefit from insights from synthetic transcription factors developed to recognize orthogonal synthetic sequences and activate gene expression (Donahue et al., 2020; Keung et al., 2014; Khalil et al., 2012). For example, the number and spacing of binding sites can be used to tune the level of gene expression (Donahue et al., 2020). In native systems, valency provides an important metric for inducing nuclear structures that are associated with transcription and splicing (Guo et al., 2019; Shrinivas et al., 2019). With increased understanding of the dynamics of chromatin structures that enable cell-fate transitions, engineered transcription factors may guide cells through rate-limiting steps in chromatin remodeling as well as target loci that remain refractory to reprogramming.

ENGINEERED BIOCHEMICAL SYSTEMS OF CHROMATIN REGULATION

Biochemical modifications of chromatin on the scale of 100 bp serve as important regulatory elements that can affect the chromatin dynamics and structure on multiple length scales (Figure 1) (Holtzman and Gersbach, 2018; Rao et al., 2014). Although biochemical marks like histone modifications correlate with specific functions (e.g., poised versus active enhancers), causal relationships remain obscured in native systems (Creyghton et al., 2010; Wapinski et al., 2013). Engineered systems of chromatin regulation offer novel opportunities to define how biochemical modifications and gene expression influence one another.

Engineered chromatin regulator (CR) proteins read, write, and erase biochemical modifications (Figure 5B). Like synthetic transcription factors, CRs activate gene expression by recruiting transcriptional machinery to specific loci to promote or interfere with biochemical modifications. For example, fusing dCas9 with CRs such as p300, BAF, or Tet1, activates gene expression from specific loci by inducing modifications such as histone acetylation (p300) and DNA demethylation (Tet1) (Braun et al., 2017; Hilton et al., 2015; Josipović et al., 2019; Liu et al., 2016). Using this approach, a myogenic enhancer was selectively demethylated via transient dCas9-Tet1 recruitment enabling reprogramming of fibroblasts into myoblasts (Liu et al., 2016). Furthermore, demethylation and successive methylation of a sialytransferase gene showed that transient CR expression can cause stable, yet reversible changes to gene expression (Marx et al., 2018).

Beyond manipulating gene expression, incorporation of native CRs into synthetic proteins and gene circuits (see Glossary) enables characterization of these elements in orthogonal contexts (Van et al., 2020). With these engineered systems, the dynamic interplay between transcription, biochemical mark placement, and mark removal can be observed (Liu et al., 2016; Molina et al., 2016). For example, integrating CRs with a reporter circuit enables tracking of biochemical modification turnover due to exposure to CRs over periods of hours to days (Bintu et al., 2016). Retention kinetics of modifications in synthetic circuits depend on the specific biochemical modification and mirror the properties observed in native systems (Bachman et al.,

(B) Fusing DNA-binding domains to native and engineered chromatin regulatory domains allows targeted modification and reading of important biochemical marks, including various histone modifications (addition or removal of acetyl and methyl marks on key residues) and DNA methylation.
(C) Insertion or deletion of SMC and CTCF-binding sites can modify loop domain dynamics, potentially affecting enhancer-promoter interactions. Modifying the spacing, direction, and adjacency of genomic elements within a contact domain can also affect gene expression.
(D) Using protein domains targeting specific loci, synthetic loop domains can be formed to increase intra-domain contacts between enclosed elements.
(E) Targeting fusion proteins to genomic loci can be used to specifically position these loci to nuclear regions, such as the nuclear lamina and Cajal bodies.
(F) Recruitment of proteins with intrinsically disordered regions (IDRs) to specific genomic loci can induce phase separation, giving a model system to investigate the role of condensates in enhancer-promoter contacts and transcriptional regulation.

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2001; Bintu et al., 2016; Guelen et al., 2008; Seki et al., 2005; Zhao et al., 2005). One study used a synthetic writer circuit and found that the boundaries of repressive heterochromatic regions were determined by a dynamic competition between mark writing and transcription-induced turnover (Figure 5B) (Hathaway et al., 2012). Biochemical marks can also directly influence RNAPII behavior; one histone acetylation mark, H3K27ac, was found to increase the rate of RNAPII elongation but does not affect the rate of RNAPII initiation (Stasevich et al., 2014). RNAPII elongation can in turn induce mark placement, highlighting the dynamic interplay at work between transcription and epigenetic modification (Morillon et al., 2005).

While engineered circuits utilizing native CRs may be affected by endogenous regulatory mechanisms, synthetic CRs provide an orthogonal alternative (Park et al., 2019; Tekel et al., 2018). Incorporation of bacterial methylation enzymes enabled construction of an orthogonal mammalian epigenetic system (Park et al., 2019). Synthetic CR enzymes methylate desired loci, read methyl marks to regulate gene expression, and passively spread existing methylation marks to adjacent unmarked chromatin. Other work has engineered and validated chromatin reader domains that recognize combinations of native chromatin marks including trimethylation of H3K4, 9, and 27 in addition to CpG DNA methylation (Villaseñor et al., 2020). Through sensing and actuation on native and synthetic biochemical signals, these CR systems may be deployed as synthetic epigenetic control systems.

Biochemical methods of marking histones provide an additional layer of control to the synthetic circuit design toolkit, enabling reversible, short-term control and long-term memory storage that can be combined within additional layers of chromatin engineering.

ENGINEERING CONTACT DOMAIN AND COMPARTMENTALIZATION MODIFICATIONS

At the largest scale of organization, the dynamic behavior of contact domains and compartments may control the communication between regions of chromatin separated by distances of more than 100 kb. In native systems, genetic editing of contact domains via CRISPR has been demonstrated to affect the activity of endogenous enhancers. Limb morphogenesis requires precise tissue-specific chromatin organization for enhancer regulation (Infante et al., 2013; Lewandowski et al., 2015; Sheth et al., 2016). For example, *Pitx1* is regulated by the *Pen* enhancer. Despite expression of Pen in both the fore and hindlimbs, *Pen* only activates *Pitx1* expression in the hindlimbs due to tissue-specific chromatin organization (Andrey et al., 2017; Kraft et al., 2015; Kragesteen et al., 2018). Flipping the orientation of the genomic region containing *Pen* brings *Pitx1-Pen* in close proximity, inducing the formation of a hindlimb-like chromatin hub including both *Pen* and *Pitx1*. Mice bearing this inversion ectopically express Pitx1 in the forelimb and develop an ectopic patella. In humans, this malformation disease is known as Liebenberg syndrome (Al-Qattan et al., 2013; Alvarado et al., 2011; Kragesteen et al., 2018). These studies highlight that chromatin structure around enhancers support complex tissue development. Additionally, genome editing enables precise investigation into the structure-function relationship of chromatin.

Genome engineering elucidates the relationship between genome structure and transcriptional dynamics including transcriptional bursting. Proximity of genes to enhancers may influence competition between genes for the same enhancer. Investigation of genes under shared enhancer control revealed that inverting one gene to place its promoter further from the enhancer caused the genes to switch from competitive transcriptional bursting to coordinated bursting (Figure 5C, right) (Fukaya et al., 2016). Another study demonstrated that deleting a contact domain boundary only affected the transcription of a gene within the domain but not outside (Figure 5C) (Yokoshi et al., 2020). Moreover, both studies found that changes in overall mRNA levels are best explained by changes in transcriptional burst frequency rather than burst size. In mammals, cohesin-mediated chromatin looping can also be modified by inserting, deleting, or inverting CTCF-binding sites (Beagan and Phillips-Cremins, 2020; de Wit et al., 2015; Jia et al., 2020). CTCF site orientation determines DNA looping and chromatin architecture and can regulate gene expression (Guo et al., 2015). Tandemly oriented CTCF-binding sites separating promoters control the relative activity of distal and proximal promoters by changing which promoter statistically prefers to dynamically contact a shared enhancer with a convergent CTCF-binding site (Jia et al., 2020). Together, these studies demonstrate that gene expression and behavior (e.g. cooperative versus competitive bursting) can be engineered through elements of chromatin structure as well as by manipulating E-P regulation via changes in dynamic chromatin structures.

While genetic modifications such as CTCF site inversion can alter statistically preferred chromatin structures, several synthetic systems induce forced looping to examine dynamics (Bartman et al., 2016; Deng et al., 2012, 2014; Kim et al., 2019b; Morgan et al., 2017). These synthetic looping systems use DNA-binding domains to target two genomic loci of interest. Fusing the DNA-binding domains to dimerization domains enables induction of forced looping upon addition of a dimerizing agent (e.g., small molecule or light) (Figure 5D) (Kim et al., 2019b; Morgan et al., 2017). Forced looping results in modest increases in gene expression. In mouse cells, induced looping increased burst fraction (related to burst frequency) but not burst size (Bartman et al., 2016). Overall, these observations suggest that gene expression is very sensitive to changes in burst frequency that may be increased by loop-mediated boundary reinforcement between enhancers and promoters.

Synthetic systems have also been developed to allow dynamic restructuring of 3D genome organization via membraneless organelles at the 100-nm to 1-μm scale. For instance, dCas9-based systems have been used to reposition specific loci to subnuclear structures (e.g., Cajal bodies [CBs], nuclear lamina), enabling repression of gene expression (Figure 5E) (Wang et al., 2018). dCas9-based systems have been shown to initiate the formation of membraneless structures like CBs and synthetic condensates at specific loci (Shin et al., 2018; Wang et al., 2018). Recently, structures formed via liquid-liquid phase separation (LLPS) have gained much attention for their ability to segregate chromatin into different phases and promote cellular processes, such as transcription, by enabling high local concentrations of transcriptional machinery (Bracha et al., 2018, 2019; Gibson et al., 2019; Hnisz et al., 2017; Shin et al., 2017). Synthetic

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condensates can mechanically restructure local chromatin organization and store memory of spatial stimuli (Figure 5F) (Dine et al., 2018; Shin et al., 2018). Although LLPS is often implicated, there exists alternate mechanisms to compartmentalization and chromatin segregation. Notably, ectopic overexpression of HP1α, a protein necessary for heterochromatin formation, results in a switch-like compaction where chromatin is either compact or decompact, existing within the same rather than separate liquid phase (Erdel et al., 2020).

Overall, condensates have been shown to alter chromatin organization and promote cellular processes like transcription, creating a feedback between structure and function. Future studies will clarify how biophysical principles of non-equilibrium processes are utilized by cells in concert with equilibrium-driven LLPS to organize the 3D genome and regulate gene expression.

RESOLVING THE INTERRELATIONSHIP BETWEEN CHROMATIN STRUCTURE AND GENOME FUNCTION

With novel tools from imaging, sequencing, genome engineering, and synthetic biology, the field of chromatin organization has witnessed accelerated discovery and innovation. Increasing levels of sophistication and resolution in imaging and sequencing methodologies have revealed increasingly detailed levels of nuclear architecture (Bintu et al., 2018; Flyamer et al., 2017; Hoffman et al., 2020; Hsieh et al., 2016; Stevens et al., 2017; Su et al., 2020). However, many open questions remain: how does transcription influence chromatin during cell-fate transitions? What are the implications for dynamic chromatin boundaries? Do enhancers directly contact promoters or do they recruit promoters to enhancer hubs? How can synthetic chromatin systems and gene circuits enable mechanistic insight into chromatin dynamics? In light of the emerging models, how do we engineer chromatin to regulate gene expression and control cell fate? A dynamic, unified view of nuclear architecture will be key both to understanding native processes and engineering novel synthetic systems (Figure 6).

RESOLVING SINGLE-CELL AND POPULATION-LEVEL ANALYSES

Most 3C-derived analyses capture the average profile of millions of cells, obscuring the relationship between cell state and chromatin dynamics. Tracking dynamics in heterogenous populations compounds the problem (Figures 6A and 6B). Single-cell imaging combined with cell-state reporters may enable more precise definition of the temporal profile of chromatin states that generates specific behaviors. For instance, high-resolution imaging methods show that contact domains do exist in single cells (Bintu et al., 2018). While single-cell domain boundaries are more heterogeneously distributed than population-averaged contact maps suggest, this indicates that contact domains identified through Hi-C are not simply artifacts of population averaging. Rather, they represent statistically preferred, coherent domain boundaries for the ensemble of dynamic chromatin states within a population (Bintu et al., 2018). Given the dynamic binding and fractional occupancy of CTCF, heterogeneous distributions should be expected (Cattoglio et al., 2019; Holzmann et al., 2019). However, these analyses do not address whether particular states (or distributions) are the result of randomness in chromatin conformations or differences in cell state such as cell-cycle phase, transcription rate, metabolism rate, or cell-fate trajectory. Integrating live or fixed readouts will enable the isolation of patterns that drive changes from those that do not. To connect chromatin dynamics with gene regulation and cell fate, synthetic systems such as cellular reprogramming provide unique contexts to study chromatin dynamics in concert with transcriptional dynamics during cell-fate transitions (Box 2). With recent developments in technologies, opportunities exist for examining chromatin dynamics with unprecedented resolution and mapping encoded sequences and structures to functional outcomes. In order to investigate transient phenomena such as enhancer-promoter contacts, methods of mapping chromatin dynamics via sequencing are emerging. Techniques such as Micro-C and Tiled-C can achieve high-resolution contact maps with only thousands of cells that may enable chromatin organization analysis of rare subpopulations of cells (Hsieh et al., 2020; Oudelaar et al., 2020).

Polymer simulations of chromatin across multiple time and length scales provide an important bridge between observations and theory (Jost et al., 2018). Simulations using Hi-C maps as constraints can successfully reproduce ensemble chromatin conformations (Beagan et al., 2020; Di Pierro et al., 2016; Di Stefano et al., 2016, 2020; Nuebler et al., 2018; Paulsen et al., 2017; Serra et al., 2017). While useful, these models use population-averaged snapshots, possibly missing dynamics present in single cells (Figures 6B and 6C). Time-point Hi-C and FISH experiments, when combined with modeling, have begun to decrease this gap (Abbas et al., 2019; Di Stefano et al., 2020). However, even detailed single-cell sequencing experiments preclude live observation and tracking. If scalable, single-cell microscopy analyses will enable the identification of causal principles and mechanisms underlying dynamic genomic architecture regulation (Su et al., 2020). Future work in modeling to bridge the divide between single-cell and population-level observations will rely on a combination of bottom-up mechanistic studies (Brackey et al., 2020) as well as integration of observations across many scales (Moller and de Pablo, 2020).

CONNECTING DYNAMICS IN CHROMATIN STRUCTURE AND GENE REGULATION

Resolving the dynamic structure-function relationship of chromatin remains a grand challenge in understanding gene regulation and resulting cell fates. Chromatin structure both affects and is affected by gene expression, presenting opportunities for redrafting the traditional arrow connecting structure to function into a feedback loop. SMC degradation studies highlight the need for revising the model of how structure supports function. For example, degradation of SMCs eliminates the formation of stable population TAD structures, but acute loss of SMCs only modestly perturbs gene expression profiles (Nora et al., 2017; Rao et al., 2017; Schwarzer et al., 2017). However, chronic loss of CTCF results in changes in proliferation and viability, making experimental inferences over physiological relevant time periods challenging (Nora et al., 2017). However, in light of massive structural changes resulting in minimal perturbations of gene expression, how do we understand the role of structure in regulating gene expression?

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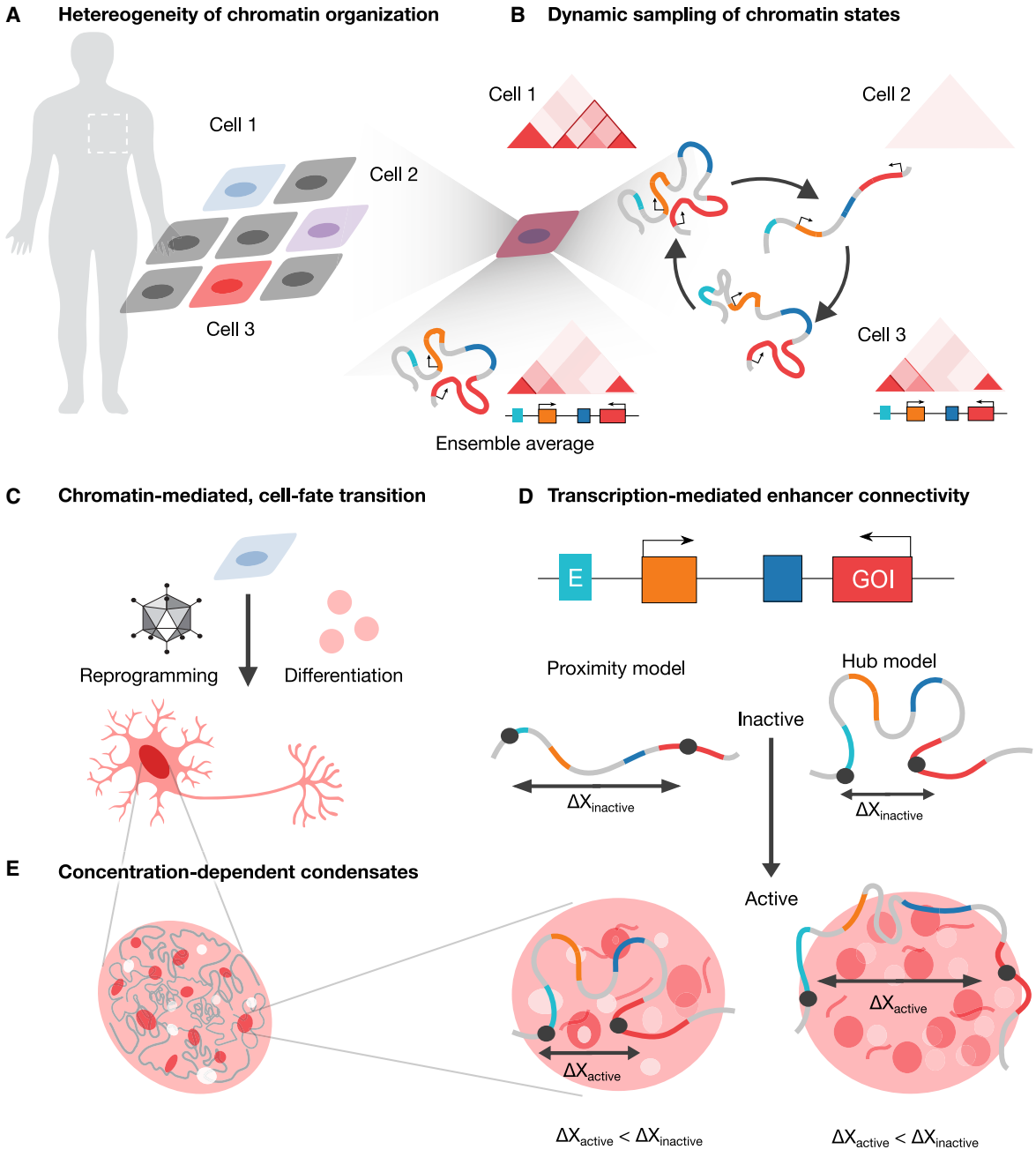

Figure 6. Resolving the Interrelationship between Chromatin Structure and Genome Function

(A) Within tissues and cell lines, cells adopt different states of chromatin organization. Gaps remain in our understanding of how heterogeneity of chromatin states varies across populations dynamically.

(B) Structures vary dynamically in response to diverse stimuli including stage of cell cycle, paracrine signaling, and interaction with the extracellular matrix.

(C) Marked by unique chromatin states, cell-fate transitions such as differentiation and reprogramming require epigenetic remodeling. Capacity for remodeling may vary dynamically by chromatin state (B).

(D) Induction of gene expression alters chromatin structure, changing the connectivity of enhancers and promoters. Two models capture diverse changes in proximity between enhancers and promoter. In the proximity model, transcriptional activity correlates with increased enhancer-promoter proximity. In the transcriptional hub model, promoter-enhancer proximity may increase as protein and RNAs are recruited to regions of active transcription.

(E) Nuclear structures associated with transcription and splicing contain high concentrations of proteins and RNAs that form phase-separated compartments called nuclear condensates.

Recent work examining loss of CTCF in reprogramming and the immune response provides some insight into how CTCF differentially impacts cell-fate transitions and cell-specific functions. Loss of CTCF does not impact B cell transdifferentiation into induced macrophages (iMacs) (Stik et al., 2020). However, when challenged with endotoxins, iMacs lacking CTCF fail to induce a full-

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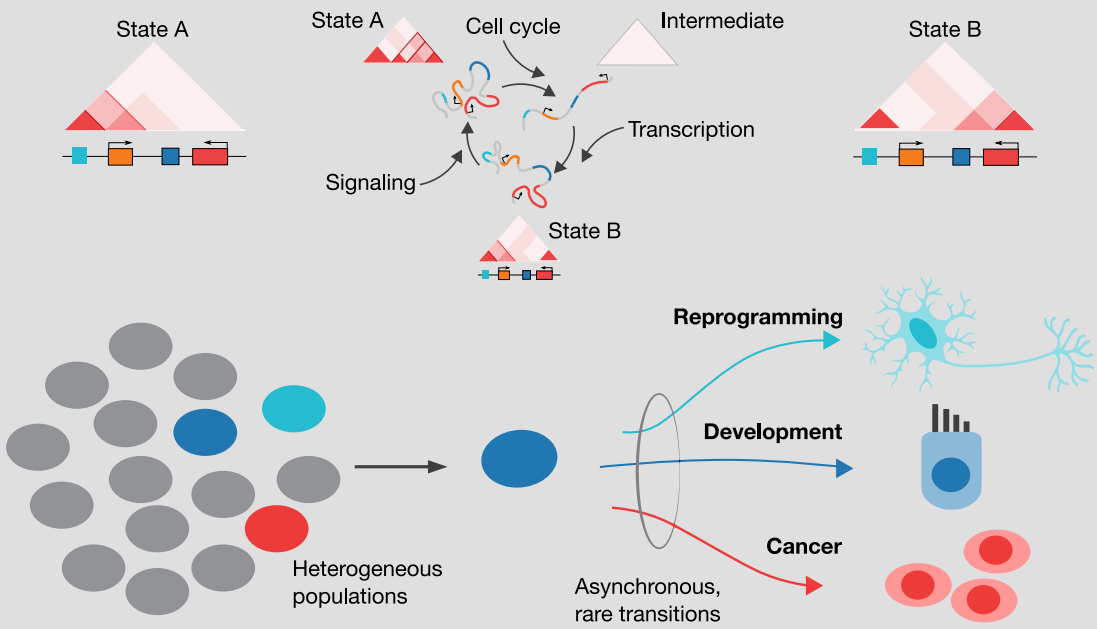
Box 2. Capturing Chromatin Dynamics during Cell-Fate Transitions



Cells integrate a vast array of external and intrinsic cues into cellular decision-making events during the transition from one identity to another. Despite these myriad of influences, emerging evidence identifies chromatin remodeling as a key step in adoption of a new cell fate. Overexpression of lineage-specifying transcription factors drives changes in domain interactions (Dall'Agnese et al., 2019; Di Giammartino et al., 2019; Stadhouders et al., 2018). Notably, chromatin rewiring in noncoding regions precedes changes in genic regions in the conversion of fibroblasts to iPSCs (Di Giammartino et al., 2019). Similar to reprogramming, transformation in cancer initiates from early epigenetic changes. Rewiring of chromatin structure precedes the induction of oncogenesis and metastasis (Denny et al., 2016; Park et al., 2018; Schuijers et al., 2018). Beyond primary tumors, dynamic epigenetic state switching enables persistence of drug-tolerant subpopulations and metastases (Denny et al., 2016; Sharma et al., 2010).

Within populations of cells, unique processes mark privileged populations capable of mediating the massive epigenetic transition to reprogram (Babos et al., 2019; Guo et al., 2014). The subpopulation of fast-cycling cells reprogram to highly proliferative iPSCs and post-mitotic neurons at much higher rates (5–100-fold) than slower cycling cells (Babos et al., 2019; Guo et al., 2014). Reprogramming of somatic nuclei via fusion with ESCs requires DNA synthesis, suggesting that S phase may provide a unique environment for reprogramming (Tsubouchi et al., 2013). Additionally, cell-cycle stage correlates with chromatin folding state (Zhang et al., 2019). Together these observations suggest that proliferation may promote reprogramming via cell-cycle-induced resetting and restructuring of chromatin. Additionally, high transcription rates in hyperproliferative cells result in near-deterministic reprogramming (Babos et al., 2019). Hypertranscription may facilitate rapid, extensive chromatin remodeling to drive cellular reprogramming. Given the rarity of privileged cells across populations, novel low-input 3C-based methods and single-cell imaging methods will be key to understanding the dynamic and highly heterogeneous processes underlying cell-fate transitions (Wang et al., 2020). Knowing how dynamic chromatin rewiring in single cells affects gene regulation may explain how transient subpopulations contribute to cell-fate transitions.

blown immune response. While large-scale structures such as TADs may be dispensable for cell-fate transitions, these structures may uniquely support temporally specific functions (e.g., infection, neuronal stimulation, and acute stress) (Cuartero et al., 2018). Thus, the deficits associated with loss of these structures would not be observed except under unique conditions, potentially explaining the lack of correlation observed between the degree of difference in structural deficits and gene expression (Nora et al., 2017; Rao et al., 2017; Schwarzer et al., 2017). The challenge remains in identifying cell- and context-specific functions of chromatin to examine this hypothesis.

If changes in the most probable large-scale structures only introduce small expression perturbations, how are smaller, more dynamic structures linked to gene regulation? One study in *Drosophila*, a species that lacks mammalian SMC-driven loop domains, integrated nascent transcript analysis with Hi-C contact data, histone mark profiling, and accessible chromatin assays and found that small (<10kb) contact domains formed based on transcriptional state (Rowley et al., 2017). In fact, profiles of nascent transcripts are sufficiently informative to recapitulate contact maps, chromatin profiles, and the strength of contact domain interactions (Rowley et al., 2017; Wang et al.,

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2020a). It has even been reported that transcription-linked chromatin reconfiguration occurs on timescales as short as neuronal stimulation (Beagan et al., 2020). In mouse embryonic stem cells (ESCs), RNAPII-mediated activity drives formation of a subset of similar small contact domains (gene-to-gene, enhancer-to-promoter) with little impact on higher-order structures such as TADs, emphasizing the role that transcription plays in establishing dynamic structures on the kilobase length scale (Hsieh et al., 2020).

If changes in transcription influence chromatin structure, how do we integrate recent work from cellular reprogramming that indicates that changes in chromatin topology precede changes in gene expression? One potential answer lies in transcription of noncoding RNAs. Transcription of noncoding regions such as lncRNAs and enhancer (eRNAs) may precede and induce topological changes in chromatin that in turn induce changes in genic, coding sequences (Klattenhoff et al., 2013; Puc et al., 2015; Stadhouders et al., 2018). Induction of androgen signaling recruits topoisomerase I to induce eRNA transcription, resulting in upregulation of coding genes (Puc et al., 2015). Additionally, RNA binding promotes the function of canonical mediators of chromatin structure. For example, CTCF binds RNA and loss of RNA-binding regions or transcriptional inhibition disrupts chromatin loops, resulting in nuclear reorganization (Hansen et al., 2019; Saldaña-Meyer et al., 2019). These studies suggest that dynamic changes in the concentrations of proteins and RNAs facilitate nuclear reorganization. Waves of transcription from noncoding to coding may guide cell-fate transitions through remodeling chromatin structures by increasing local concentration of RNAs and inducing mechanical changes in chromatin state.

Chromatin states at smaller scales are significantly impacted by transcription-mediated mechanical epigenetics (Box 1). Recent experiments have started to reveal a rich relationship between transcription and nucleosome positioning and the underlying mechanical state of DNA (Hsieh et al., 2020; Naughton et al., 2013). These phenomena collectively indicate the ability of DNA-bound processes to influence chromatin organization, leading to a fundamental connection between chromosome function and structure. Resolving the impact of feedback between transcription-generated structure on subsequent transcriptional dynamics may illuminate principles for engineering transcriptional regulation via genetically encoded dynamic structures. While some computational work has been done to reconcile observations of chromatin dynamics (Khanna et al., 2019; Lampo et al., 2016; Di Pierro et al., 2018; Polovnikov et al., 2018), future work is needed to fully connect the dynamics of chromatin function and structure (Brackey et al., 2020).

UNDERSTANDING MODES OF ENHANCER REGULATION

Enhancers confer cell-type specificity by maintaining the unique expression profile that characterizes each cell type and confers unique sensitivity during development and disease (Furlong and Levine, 2018; Lu et al., 2020; Sakabe et al., 2012). During differentiation, chromatin remodeling enables enhancer engagement with promoters to initiate transcription of gene regulatory networks that confer a new cell identity. However, the mechanisms by which engagement occurs and how enhancers influence chromatin structure and dynamics to confer cell identity remains poorly understood (Di Giammartino et al., 2019; Mateo et al., 2019). Though a consensus perspective has yet to emerge, enhancers are traditionally defined as noncoding regulatory sequences of DNA that can increase expression of genes via recruitment of transcriptional machinery to promoters (Pennacchio et al., 2013). A subset of enhancers induce transcription through a proximity-based model that requires contact with cognate promoters (Chen et al., 2018); others act in a proximity-independent fashion (Figures 6D and 6E) (Benabdallah et al., 2019). Proximity-independent models suggest that enhancers may induce promoter activity through distinct mechanisms including noncoding transcription at enhancers, the direct alteration of chromatin structure, scaffolding of regulatory elements, and the formation of condensates (Bracha et al., 2019; Shin et al., 2018; Shrinivas et al., 2019; Zamudio et al., 2019). These mechanisms recruit transcriptional regulators including RNA, mediator, and RNAPII, which in turn increase the local concentration of transcriptional machinery at enhancers and distally located promoters (Cho et al., 2018; Shrinivas et al., 2019; Zamudio et al., 2019). By forming subnuclear regions with high protein and RNA concentration, these biomolecular condensates (sometimes called "hubs") may enable indirect enhancer-promoter regulation across larger distances (e.g., up to a micron) (Benabdallah et al., 2019; Cho et al., 2018; Zamudio et al., 2019). How these condensates form, shape chromatin, and regulate gene expression via their interactions with enhancers remains an active area of research.

Beyond impacting supercoiling structures, transcription at enhancers may impact chromatin organization by increasing the local concentration of RNA. Emerging evidence suggests noncoding RNAs shape chromatin structure (Nozawa and Gilbert, 2019). Loss of noncoding RNAs reduces differentiation capacity of ESCs toward different lineages, suggesting locus-specific effects for noncoding RNAs that contribute to adoption of new transcriptional states (Guttman et al., 2011). Improved imaging and sequencing methods, as well as live-cell tracking of identity and transcription, will connect transcription to enhancer activity in single cells, clarifying the mechanism by which enhancers enable transcriptional control as well as highlighting fragile regulatory points sensitive to local perturbations.

Additional regulatory elements that may function similarly to enhancers include insulators and silencers. Insulators were originally recognized as elements that blocked heterochromatin mark spreading and have now also been implicated in the formation of some contact domain boundaries (Khurana et al., 2016; Yang and Corces, 2011). Identified insulator regions include the binding sites of architectural proteins such as CTCF, the binding sites of other DNA-binding proteins, and certain highly expressed tRNA genes (Chen and Corces, 2001; Hou et al., 2012; Liao et al., 2018). Silencers actively decrease transcription of encoded genes (Pang and Snyder, 2020). While some well-known insulators and silencers such as CTCF and Polycomb have been characterized, the mechanistic function and genomic distribution of this class of elements are not fully understood (Cao et al., 2002; Fukaya et al., 2016; Pang and Snyder, 2020).

CONCLUDING THOUGHTS

Combining synthetic bottom-up approaches with top-down genome engineering, novel imaging and sequencing techniques

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will improve the molecular detail with which we understand the role of chromatin regulation on gene expression and cellular behavior. With a wealth of new tools, our ability to understand the interrelationship between chromatin structure and function will rapidly accelerate through the thoughtful integration of methods and approaches. Already new tools highlight transcription as a key regulator of chromatin dynamics and structure. In the future, as our understanding of how transcription shapes chromatin in native systems expands, synthetic biologists will apply this understanding to develop strategies to sense and control chromatin structures to regulate gene expression. By harnessing an important layer of native gene regulation, engineering chromatin-mediated control of gene expression will improve the reliability of gene- and cell-based therapies.

GLOSSARY

A and B Compartments
Originating from PCA of early Mb-sized compartments, the A and B naming convention is used to refer to transcriptionally active and inactive regions, which are now known to go down to the kb scale and result in checkerboard patterns on genome-wide contact maps (related: compartmental domains, eigenvector, microphase separation).

Bottom-up
"Bottom-up" approaches involve forward engineering of systems to achieve specific design objectives. Bottom-up approaches may include addition of native or synthetic binding sites, inclusion of larger noncoding regulatory sequences, design of synthetic regulatory regions that insulate or interact with native chromatin structures.

Chromatin
The most elementary component of genetic material in eukaryotic organisms consisting primarily of DNA and histones. It serves as the first layer of DNA organization whereby genetic information stored in the dsDNA base pairs is dynamically wrapped around histones to form nucleosomes. The basic composition of chromatin (such as where nucleosomes exist and their affinity for DNA) serves as the first layer of epigenetic regulation, influencing the local steric and mechanical state of DNA near important areas such as transcription start sites. Additionally, chromatin serves as the polymeric substance used to form higher-order chromosomal structures.

Compartmental Domain
Large-Mb-scale A/B compartments are actually composed of much finer ~5 kb compartmental domains that can only be detected by high-resolution contact maps.

Contact Domain
A type of chromatin organization where certain genomic regions experience increased physical proximity resulting in decreased spatial distances and increased chromatin contact frequency. They are manifested as on-diagonal triangles in Hi-C maps (related: loop domain, TAD).

Corner Dot
A punctate cluster of significantly increased contact frequencies relative to neighboring loci, appearing as a dot at the corner of triangle domains in Hi-C contact maps. Contact domains with corner dots are typically annotated as loop domains.

CTCF
CCCTC-binding factor (CTCF) is an architectural protein with multiple regulatory roles and blocks loop extrusion when bound to convergently oriented CTCF-binding sites in mammals.

dCas9
A nuclease-deficient Cas9 enzyme that is capable of recognizing guide-RNA-encoded targets but cannot introduce DNA strand breaks. When combined with other techniques, dCas9 can be used to recruit various fluorescent probes to specific genomic loci.

Diffraction-Limited
Imaging techniques where the maximum resolution is determined by the physical spreading of a point source signal due to diffraction.

DNA Supercoiling
The over or under winding of DNA resulting in local and global changes in DNA topology and mechanics. Active processes such as transcription will both influence it due to mechanical coupling between elongation and DNA rotation, as well as be influenced by it due to altered transcription initiation and elongation bubble stability (see Box 1).

Eigenvector
Eigenvectors give underlying information of a system assuming it behaves linearly. The principal component in PCA is simply an eigenvector of the covariance matrix, which can be used to assign A and B compartments.

FISH
FISH, a method for fluorescently tagging specific DNA or RNA targets.

Histone
Fundamental protein complexes found in eukaryotes. Through various biochemical modifications they display variable affinity for having DNA wrapped around them, forming nucleosomes.

In Situ Proximity Ligation
An improved version of proximity ligation where the ligation step is performed in intact crosslinked nuclei to reduce noise, replacing older protocols which lysed nuclei open and diluted crosslinked chromatin prior to ligation (related: 3C-based methods).

k_bT Energy
A unit of energy frequently used to scale energies of single molecules. Watson-crick base pairing is roughly ~2.5 to 4 k_bT.

Level of Resolution
Contact frequencies are measured by sequencing proximity ligation products and binning them into regions of DNA, where the

bin size is the level of resolution of the contact map (related: 3C-based methods).

Loop Domain
A type of contact domain resulting from loop extrusion, usually indicated by the existence of a TAD which has strong point-like corners in Hi-C (related: loop extrusion, corner dots).

Loop Extrusion
The process by which chromatin is expelled from active anchoring factors to form loops.

Microphase Separation
The underlying physical mechanism thought to be responsible for the formation of compartments, whereby active and inactive regions of chromatin form copolymer structures, which physically segregate due to a number of potential mechanisms.

Micro-C
The highest resolution version of Hi-C where a micrococcal nuclease digest is used to generate the DNA fragments, yielding evenly spaced mononucleosomes and thus 200-bp contact map resolution.

Oligopaint
A technique used for creating a fluorescently labeled heterogenous DNA or RNA probe library.

RNA Polymerase II (RNAPII)
Eukaryotic RNA polymerase responsible for transcription of coding genes. RNAPII transcripts include a 5′ cap and poly-adenylation tail.

Structural Maintenance Complexes (SMCs)
A family of ATP-consuming protein structures that is responsible for many aspects of chromatin organization including the formation of the mitotic chromosomal as well as loop extruding factors

Super-Resolution
Imaging techniques where point sources are only transiently active, which allow for precise calculation of the center of a diffracted point source.

Synthetic Biology
The systematic engineering of native biological systems to extract design principles (e.g., build-to-understand) as well as the forward design of novel, synthetic tools, devices, and systems. More simply, synthetic biology can be defined as "engineering biology" with a special emphasis on molecular-scale engineering (related: bottom-up).

Synthetic Gene Circuit
Interlinked synthetic transcriptional networks regulating gene expression.

TALE (Transcription Activator-Like Effectors):
Proteins that contain subdomains that specifically bind to single base pairs. Multiple subdomains can be combined in a fusion protein in order to recognize genomic loci with base pair specificity

Topologically Associated Domain (TAD)
A type of contact domain that occurs on the genomic scale of 100 kb.

Top-Down
"Top-down" approaches involve reverse-engineering of systems by systematic subtraction of elements followed by characterization of function. From a biological perspective, top-down approaches may involve loss of function mutant, deletion of protein domains, or removal of DNA-binding sites.

Transcriptional Element
A single component that is transcribed. Synthetic transcriptional elements often include a regulatory element (e.g., promoter), transcription start site, and gene (e.g., coding or noncoding) and poly-adenylation sequence.

3C-Based Methods
The pioneering 3C method uses proximity ligation to quantify how frequently 2 genomic loci are close together in a "one-to-one" manner. Subsequent derived methods have expanded how many pairwise contacts can be simultaneously probed, such as 4C ("one to all"), 5C ("many to many"), and Hi-C ("all to all") and are collectively called 3C-based methods (related: *in situ* proximity ligation, level of resolution).

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REFERENCES

Abbas, A., He, X., Niu, J., Zhou, B., Zhu, G., Ma, T., Song, J., Gao, J., Zhang, M.Q., and Zeng, J. (2019). Integrating Hi-C and FISH data for modeling of the 3D organization of chromosomes. Nat. Commun. *10*, 2049.

Achar, Y.J., Adhil, M., Choudhary, R., Gilbert, N., and Foiani, M. (2020). Negative supercoil at gene boundaries modulates gene topology. Nature *577*, 701–705.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). Chromosomal DNA and its packaging in the chromatin fiber. In Molecular Biology of the Cell, Fourth Edition (Routledge), pp. 173–236. https://www.ncbi.nlm.nih.gov/books/NBK26834/.

Al-Qattan, M.M., Al-Thunayan, A., AlAbdulkareem, I., and Al Balwi, M. (2013). Liebenberg syndrome is caused by a deletion upstream to the PITX1 gene resulting in transformation of the upper limbs to reflect lower limb characteristics. Gene *524*, 65–71.

Alvarado, D.M., McCall, K., Aferol, H., Silva, M.J., Garbow, J.R., Spees, W.M., Patel, T., Siegel, M., Dobbs, M.B., and Gurnett, C.A. (2011). Pitx1 haploinsufficiency causes clubfoot in humans and a clubfoot-like phenotype in mice. Hum. Mol. Genet. *20*, 3943–3952.

Andrey, G., Schöpflin, R., Jerković, I., Heinrich, V., Ibrahim, D.M., Paliou, C., Hochradel, M., Timmermann, B., Haas, S., Vingron, M., and Mundlos, S. (2017). Characterization of hundreds of regulatory landscapes in developing limbs reveals two regimes of chromatin folding. Genome Res. *27*, 223–233.

Babos, K.N., Galloway, K.E., Kisler, K., Zitting, M., Li, Y., Shi, Y., Quintino, B., Chow, R.H., Zlokovic, B.V., and Ichida, J.K. (2019). Mitigating antagonism

between transcription and proliferation allows near-deterministic cellular reprogramming. Cell Stem Cell *25*, 486–500.e9.

Bachman, K.E., Rountree, M.R., and Baylin, S.B. (2001). Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. J. Biol. Chem. *276*, 32282–32287.

Balboa, D., Weltner, J., Eurola, S., Trokovic, R., Wartiovaara, K., and Otonkoski, T. (2015). Conditionally stabilized dCas9 activator for controlling gene expression in human cell reprogramming and differentiation. Stem Cell Reports *5*, 448–459.

Bancaud, A., Conde e Silva, N., Barbi, M., Wagner, G., Allemand, J.F., Mozziconacci, J., Lavelle, C., Croquette, V., Victor, J.M., Prunell, A., and Viovy, J.L. (2006). Structural plasticity of single chromatin fibers revealed by torsional manipulation. Nat. Struct. Mol. Biol. *13*, 444–450.

Baranello, L., Levens, D., and Kouzine, F. (2018). 3.DNA supercoiling(omics). In Nuclear Architecture and Dynamics, C. Lavelle and J.-M. Victor, eds. (Academic Press), pp. 81–99.

Bartman, C.R., Hsu, S.C., Hsiung, C.C.-S., Raj, A., and Blobel, G.A. (2016). Enhancer regulation of transcriptional bursting parameters revealed by forced chromatin looping. Mol. Cell *62*, 237–247.

Beagan, J.A., Pastuzyn, E.D., Fernandez, L.R., Guo, M.H., Feng, K., Titus, K.R., Chandrashekar, H., Shepherd, J.D., and Phillips-Cremins, J.E. (2020). Three-dimensional genome restructuring across timescales of activity-induced neuronal gene expression. Nat. Neurosci. *23*, 707–717.

Beagan, J.A., and Phillips-Cremins, J.E. (2020). On the existence and functionality of topologically associating domains. Nat. Genet. *52*, 8–16.

Beagrie, R.A., Scialdone, A., Schueler, M., Kraemer, D.C.A., Chotalia, M., Xie, S.Q., Barbieri, M., de Santiago, I., Lavitas, L.M., Branco, M.R., et al. (2017). Complex multi-enhancer contacts captured by genome architecture mapping. Nature *543*, 519–524.

Beagrie, R.A., Thieme, C.J., Annunziatella, C., Baugher, C., Zhang, Y., Schueler, M., Kramer, D.C., Chiariello, A.M., Bianco, S., Kukalev, A., et al. (2020). Multiplex-GAM: genome-wide identification of chromatin contacts yields insights not captured by Hi-C. bioRxiv. https://doi.org/10.1101/2020.07.31.230284.

Beliveau, B.J., Apostolopoulos, N., and Wu, C.-T. (2014). Visualizing genomes with Oligopaint FISH probes. Curr. Protoc. Mol. Biol. *105*, 14.23.1–14.23.20.

Beliveau, B.J., Boettiger, A.N., Avendaño, M.S., Jungmann, R., McCole, R.B., Joyce, E.F., Kim-Kiselak, C., Bantignies, F., Fonseka, C.Y., Erceg, J., et al. (2015). Single-molecule super-resolution imaging of chromosomes and in situ haplotype visualization using Oligopaint FISH probes. Nat. Commun. *6*, 7147.

Beliveau, B.J., Joyce, E.F., Apostolopoulos, N., Yilmaz, F., Fonseka, C.Y., McCole, R.B., Chang, Y., Li, J.B., Senaratne, T.N., Williams, B.R., et al. (2012). Versatile design and synthesis platform for visualizing genomes with Oligopaint FISH probes. Proc. Natl. Acad. Sci. USA *109*, 21301–21306.

Benabdallah, N.S., Williamson, I., Illingworth, R.S., Kane, L., Boyle, S., Sengupta, D., Grimes, G.R., Therizols, P., and Bickmore, W.A. (2019). Decreased enhancer-promoter proximity accompanying enhancer activation. Mol. Cell *76*, 473–484.e7.

Bintu, B., Mateo, L.J., Su, J.H., Sinnott-Armstrong, N.A., Parker, M., Kinrot, S., Yamaya, K., Boettiger, A.N., and Zhuang, X. (2018). Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. Science *362*, eaau1783.

Bintu, L., Yong, J., Antebi, Y.E., McCue, K., Kazuki, Y., Uno, N., Oshimura, M., and Elowitz, M.B. (2016). Dynamics of epigenetic regulation at the single-cell level. Science *351*, 720–724.

Black, J.B., Adler, A.F., Wang, H.G., D'Ippolito, A.M., Hutchinson, H.A., Reddy, T.E., Pitt, G.S., Leong, K.W., and Gersbach, C.A. (2016). Targeted epigenetic remodeling of endogenous loci by CRISPR/Cas9-based transcriptional activators directly converts fibroblasts to neuronal cells. Cell Stem Cell *19*, 406–414.

Bonev, B., Mendelson Cohen, N., Szabo, Q., Fritsch, L., Papadopoulos, G.L., Lubling, Y., Xu, X., Lv, X., Hugnot, J.P., Tanay, A., and Cavalli, G. (2017). Multiscale 3D genome rewiring during mouse neural development. Cell *171*, 557–572.e24.

Bracha, D., Walls, M.T., and Brangwynne, C.P. (2019). Probing and engineering liquid-phase organelles. Nat. Biotechnol. *37*, 1435–1445.

Bracha, D., Walls, M.T., Wei, M.-T., Zhu, L., Kurian, M., Avalos, J.L., Toettcher, J.E., and Brangwynne, C.P. (2018). Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds. Cell *175*, 1467–1480.e13.

Brackey, C.A., Marenduzzo, D., and Gilbert, N. (2020). Mechanistic modeling of chromatin folding to understand function. Nat. Methods *17*, 767–775.

Brandão, H.B., Paul, P., van den Berg, A.A., Rudner, D.Z., Wang, X., and Mirny, L.A. (2019). RNA polymerases as moving barriers to condensin loop extrusion. Proc. Natl. Acad. Sci. USA *116*, 20489–20499.

Braun, S.M.G., Kirkland, J.G., Chory, E.J., Husmann, D., Calarco, J.P., and Crabtree, G.R. (2017). Rapid and reversible epigenome editing by endogenous chromatin regulators. Nat. Commun. *8*, 560.

Cahan, P., Li, H., Morris, S.A., Lummertz da Rocha, E.L., Daley, G.Q., and Collins, J.J. (2014). CellNet: network biology applied to stem cell engineering. Cell *158*, 903–915.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S., and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science *298*, 1039–1043.

Cattoglio, C., Pustova, I., Walther, N., Ho, J.J., Hantsche-Grininger, M., Inouye, C.J., Hossain, M.J., Dailey, G.M., Ellenberg, J., Darzacq, X., et al. (2019). Determining cellular CTCF and cohesin abundances to constrain 3D genome models. eLife *8*, e40164.

Chakraborty, S., Ji, H., Kabadi, A.M., Gersbach, C.A., Christoforou, N., and Leong, K.W. (2014). A CRISPR/Cas9-based system for reprogramming cell lineage specification. Stem Cell Reports *3*, 940–947.

Chavez, A., Tuttle, M., Pruitt, B.W., Ewen-Campen, B., Chari, R., Ter-Ovanesyan, D., Haque, S.J., Cecchi, R.J., Kowal, E.J.K., Buchthal, J., et al. (2016). Comparison of Cas9 activators in multiple species. Nat. Methods *13*, 563–567.

Chen, B., Gilbert, L.A., Cimini, B.A., Schnitzbauer, J., Zhang, W., Li, G.W., Park, J., Blackburn, E.H., Weissman, J.S., Qi, L.S., and Huang, B. (2013). Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. Cell *155*, 1479–1491.

Chen, H., Levo, M., Barinov, L., Fujioka, M., Jaynes, J.B., and Gregor, T. (2018). Dynamic interplay between enhancer-promoter topology and gene activity. Nat. Genet. *50*, 1296–1303.

Chen, S., and Corces, V.G. (2001). The gypsy insulator of Drosophila affects chromatin structure in a directional manner. Genetics *159*, 1649–1658.

Cho, W.K., Spille, J.H., Hecht, M., Lee, C., Li, C., Grube, V., and Cisse, I.I. (2018). Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science *361*, 412–415.

Corless, S., and Gilbert, N. (2016). Effects of DNA supercoiling on chromatin architecture. Biophys. Rev. *8*, 245–258.

Corless, S., Naughton, C., and Gilbert, N. (2014). Profiling DNA supercoiling domains in vivo. Genomics Data *2*, 264–267.

Creyghton, M.P., Cheng, A.W., Welstead, G.G., Kooistra, T., Carey, B.W., Steine, E.J., Hanna, J., Lodato, M.A., Frampton, G.M., Sharp, P.A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc. Natl. Acad. Sci. USA *107*, 21931–21936.

Cuartero, S., Weiss, F.D., Dharmalingam, G., Guo, Y., Ing-Simmons, E., Masella, S., Robles-Rebollo, I., Xiao, X., Wang, Y.F., Barozzi, I., et al. (2018). Control of inducible gene expression links cohesin to hematopoietic progenitor self-renewal and differentiation. Nat. Immunol. *19*, 932–941.

Dall'Agnese, A., Caputo, L., Nicoletti, C., di Iulio, J., Schmitt, A., Gatto, S., Diao, Y., Ye, Z., Forcato, M., Perera, R., et al. (2019). Transcription factor-Directed Re-wiring of chromatin architecture for somatic cell nuclear reprogramming toward trans-differentiation. Mol. Cell *76*, 453–472.e8.

de Wit, E., Vos, E.S.M., Holwerda, S.J.B., Valdes-Quezada, C., Verstegen, M.J.A.M., Teunissen, H., Splinter, E., Wijchers, P.J., Krijger, P.H.L., and de Laat, W. (2015). CTCF binding polarity determines chromatin looping. Mol. Cell *60*, 676–684.

Dekker, J., and Mirny, L. (2016). The 3D genome as moderator of chromosomal communication. Cell *164*, 1110–1121.

Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002). Capturing chromosome conformation. Science *295*, 1306–1311.

Deng, W., Lee, J., Wang, H., Miller, J., Reik, A., Gregory, P.D., Dean, A., and Blobel, G.A. (2012). Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. Cell *149*, 1233–1244.

Deng, W., Rupon, J.W., Krivega, I., Breda, L., Motta, I., Jahn, K.S., Reik, A., Gregory, P.D., Rivella, S., Dean, A., and Blobel, G.A. (2014). Reactivation of developmentally silenced globin genes by forced chromatin looping. Cell *158*, 849–860.

Denker, A., and de Laat, W. (2016). The second decade of 3C technologies: detailed insights into nuclear organization. Genes Dev. *30*, 1357–1382.

Denny, S.K., Yang, D., Chuang, C.H., Brady, J.J., Lim, J.S., Grüner, B.M., Chiou, S.H., Schep, A.N., Baral, J., Hamard, C., et al. (2016). Nfib promotes metastasis through a widespread increase in chromatin accessibility. Cell *166*, 328–342.

Desai, R.V., Hansen, M.M.K., Martin, B., Yu, C., Ding, S., Thomson, M., and Weinberger, L.S. (2020). Discovery of a cellular mechanism regulating transcriptional noise. bioRxiv. https://doi.org/10.1101/2020.06.29.128439.

Di Giammartino, D.C., Kloetgen, A., Polyzos, A., Liu, Y., Kim, D., Murphy, D., Abuhashem, A., Cavaliere, P., Aronson, B., Shah, V., et al. (2019). KLF4 is involved in the organization and regulation of pluripotency-associated three-dimensional enhancer networks. Nat. Cell Biol. *21*, 1179–1190.

Di Pierro, M., Zhang, B., Aiden, E.L., Wolynes, P.G., and Onuchic, J.N. (2016). Transferable model for chromosome architecture. Proc. Natl. Acad. Sci. USA *113*, 12168–12173.

Di Pierro, M.D., Potoyan, D.A., Wolynes, P.G., and Onuchic, J.N. (2018). Anomalous diffusion, spatial coherence, and viscoelasticity from the energy landscape of human chromosomes. Proc. Natl. Acad. Sci. USA *115*, 7753–7758.

Di Stefano, M., Paulsen, J., Lien, T.G., Hovig, E., and Micheletti, C. (2016). Hi-C-constrained physical models of human chromosomes recover functionally-related properties of genome organization. Sci. Rep. *6*, 35985.

Di Stefano, M., Stadhouders, R., Farabella, I., Castillo, D., Serra, F., Graf, T., and Marti-Renom, M.A. (2020). Transcriptional activation during cell reprogramming correlates with the formation of 3D open chromatin hubs. Nat. Commun. *11*, 2564.

Dine, E., Gil, A.A., Uribe, G., Brangwynne, C.P., and Toettcher, J.E. (2018). Protein phase separation provides long-term memory of transient spatial stimuli. Cell Syst. *6*, 655–663.e5.

Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature *485*, 376–380.

Donahue, P.S., Draut, J.W., Muldoon, J.J., Edelstein, H.I., Bagheri, N., and Leonard, J.N. (2020). The comet toolkit for composing customizable genetic programs in mammalian cells. Nat. Commun. *11*, 779.

Dostie, J., Richmond, T.A., Arnaout, R.A., Selzer, R.R., Lee, W.L., Honan, T.A., Rubio, E.D., Krumm, A., Lamb, J., Nusbaum, C., et al. (2006). Chromosome conformation capture carbon copy (5C): a massively parallel solution for mapping interactions between genomic elements. Genome Res. *16*, 1299–1309.

Eguchi, A., Wleklinski, M.J., Spurgat, M.C., Heiderscheit, E.A., Kropornicka, A.S., Vu, C.K., Bhimsaria, D., Swanson, S.A., Stewart, R., Ramanathan, P., et al. (2016). Reprogramming cell fate with a genome-scale library of artificial transcription factors. Proc. Natl. Acad. Sci. USA *113*, E8257–E8266.

El Houdaigui, B., Forquet, R., Hindré, T., Schneider, D., Nasser, W., Reverchon, S., and Meyer, S. (2019). Bacterial genome architecture shapes global transcriptional regulation by DNA supercoiling. Nucleic Acids Res. *47*, 5648–5657.

Erdel, F., Rademacher, A., Vlijm, R., Tünnermann, J., Frank, L., Weinmann, R., Schweigert, E., Yserentant, K., Hummert, J., Bauer, C., et al. (2020). Mouse heterochromatin adopts digital compaction states without showing hallmarks of HP1-driven liquid-liquid phase separation. Mol. Cell *78*, 236–249.e7.

Fierz, B., and Poirier, M.G. (2019). Biophysics of chromatin dynamics. Annu. Rev. Biophys. *48*, 321–345.

Fiorillo, L., Musella, F., Kempfer, R., Chiariello, A.M., Bianco, S., Kukalev, A., Irastorza-Azcarate, I., Esposito, A., Conte, M., Prisco, A., et al. (2020). Comparison of the Hi-C, GAM and Sprite methods by use of polymer models of chromatin. bioRxiv. https://doi.org/10.1101/2020.04.24.059915.

Flitsch, L.J., Laupman, K.E., and Brüstle, O. (2020). Transcription factor-based fate specification and forward programming for neural regeneration. Front. Cell. Neurosci. *14*, 121.

Flyamer, I.M., Gassler, J., Imakaev, M., Brandão, H.B., Ulianov, S.V., Abdennur, N., Razin, S.V., Mirny, L.A., and Tachibana-Konwalski, K. (2017). Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. Nature *544*, 110–114.

Fudenberg, G., Imakaev, M., Lu, C., Goloborodko, A., Abdennur, N., and Mirny, L.A. (2016). Formation of chromosomal domains by loop extrusion. Cell Rep. *15*, 2038–2049.

Fukaya, T., Lim, B., and Levine, M. (2016). Enhancer control of transcriptional bursting. Cell *166*, 358–368.

Furlong, E.E.M., and Levine, M. (2018). Developmental enhancers and chromosome topology. Science *361*, 1341–1345.

Germier, T., Kocanova, S., Walther, N., Bancaud, A., Shaban, H.A., Sellou, H., Politi, A.Z., Ellenberg, J., Gallardo, F., and Bystricky, K. (2017). Real-time imaging of a single gene reveals transcription-initiated local confinement. Biophys. J. *113*, 1383–1394.

Gibson, B.A., Doolittle, L.K., Schneider, M.W.G., Jensen, L.E., Gamarra, N., Henry, L., Gerlich, D.W., Redding, S., and Rosen, M.K. (2019). Organization of chromatin by intrinsic and regulated phase separation. Cell *179*, 470–484.e21.

Giepmans, B.N.G., Adams, S.R., Ellisman, M.H., and Tsien, R.Y. (2006). The fluorescent toolbox for assessing protein location and function. Science *312*, 217–224.

Gilbert, L.A., Horlbeck, M.A., Adamson, B., Villalta, J.E., Chen, Y., Whitehead, E.H., Guimaraes, C., Panning, B., Ploegh, H.L., Bassik, M.C., et al. (2014). Genome-scale CRISPR-mediated control of gene repression and activation. Cell *159*, 647–661.

Gu, B., Swigut, T., Spencley, A., Bauer, M.R., Chung, M., Meyer, T., and Wysocka, J. (2018). Transcription-coupled changes in nuclear mobility of mammalian cis-regulatory elements. Science *359*, 1050–1055.

Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de Klein, A., Wessels, L., de Laat, W., and van Steensel, B. (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature *453*, 948–951.

Guo, S., Zi, X., Schulz, V.P., Cheng, J., Zhong, M., Koochaki, S.H.J., Megyola, C.M., Pan, X., Heydari, K., Weissman, S.M., et al. (2014). Nonstochastic reprogramming from a privileged somatic cell state. Cell *156*, 649–662.

Guo, Y., Xu, Q., Canzio, D., Shou, J., Li, J., Gorkin, D.U., Jung, I., Wu, H., Zhai, Y., Tang, Y., et al. (2015). CRISPR inversion of CTCF sites alters genome topology and enhancer/promoter function. Cell *162*, 900–910.

Guo, Y.E., Manteiga, J.C., Henninger, J.E., Sabari, B.R., Dall'Agnese, A., Hannett, N.M., Spille, J.H., Afeyan, L.K., Zamudio, A.V., Shrinivas, K., et al. (2019). Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. Nature *572*, 543–548.

Guttman, M., Donaghey, J., Carey, B.W., Garber, M., Grenier, J.K., Munson, G., Young, G., Lucas, A.B., Ach, R., Bruhn, L., et al. (2011). lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature *477*, 295–300.

Gwosch, K.C., Pape, J.K., Balzarotti, F., Hoess, P., Ellenberg, J., Ries, J., and Hell, S.W. (2020). MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells. Nat. Methods *17*, 217–224.

Hansen, A.S., Hsieh, T.-H.S., Cattoglio, C., Pustova, I., Saldaña-Meyer, R., Reinberg, D., Darzacq, X., and Tjian, R. (2019). Distinct classes of chromatin loops revealed by deletion of an RNA-binding region in CTCF. Mol. Cell *76*, 395–411.e13.

Hansen, A.S., Pustova, I., Cattoglio, C., Tjian, R., and Darzacq, X. (2017). CTCF and cohesin regulate chromatin loop stability with distinct dynamics. eLife *6*, e25776.

Hathaway, N.A., Bell, O., Hodges, C., Miller, E.L., Neel, D.S., and Crabtree, G.R. (2012). Dynamics and memory of heterochromatin in living cells. Cell *149*, 1447–1460.

Hilton, I.B., D'Ippolito, A.M., Vockley, C.M., Thakore, P.I., Crawford, G.E., Reddy, T.E., and Gersbach, C.A. (2015). Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat. Biotechnol. *33*, 510–517.

Hnisz, D., Shrinivas, K., Young, R.A., Chakraborty, A.K., and Sharp, P.A. (2017). A phase separation model for transcriptional control. Cell *169*, 13–23.

Hoffman, D.P., Shtengel, G., Xu, C.S., Campbell, K.R., Freeman, M., Wang, L., Milkie, D.E., Pasolli, H.A., Iyer, N., Bogovic, J.A., et al. (2020). Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. Science *367*.

Holtzman, L., and Gersbach, C.A. (2018). Editing the epigenome: reshaping the genomic landscape. Annu. Rev. Genomics Hum. Genet. *19*, 43–71.

Holzmann, J., Politi, A.Z., Nagasaka, K., Hantsche-Grininger, M., Walther, N., Koch, B., Fuchs, J., Dürnberger, G., Tang, W., Ladurner, R., et al. (2019). Absolute quantification of cohesin, CTCF and their regulators in human cells. eLife *8*, e46269.

Hou, C., Li, L., Qin, Z.S., and Corces, V.G. (2012). Gene density, transcription, and insulators contribute to the partition of the Drosophila genome into physical domains. Mol. Cell *48*, 471–484.

Hsieh, T.-H.S., Cattoglio, C., Slobodyanyuk, E., Hansen, A.S., Rando, O.J., Tjian, R., and Darzacq, X. (2020). Resolving the 3D landscape of transcription-linked mammalian chromatin folding. Mol. Cell *78*, 539–553.e8.

Hsieh, T.-H.S., Fudenberg, G., Goloborodko, A., and Rando, O.J. (2016). Micro-C XL: assaying chromosome conformation from the nucleosome to the entire genome. Nat. Methods *13*, 1009–1011.

Ieda, M., Fu, J.D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell *142*, 375–386.

Infante, C.R., Park, S., Mihala, A.G., Kingsley, D.M., and Menke, D.B. (2013). Pitx1 broadly associates with limb enhancers and is enriched on hindlimb cis-regulatory elements. Dev. Biol. *374*, 234–244.

Jia, Z., Li, J., Ge, X., Wu, Y., Guo, Y., and Wu, Q. (2020). Tandem CTCF sites function as insulators to balance spatial chromatin contacts and topological enhancer-promoter selection. Genome Biol. *21*, 75.

Josipović, G., Tadić, V., Klasić, M., Zanki, V., Bečeheli, I., Chung, F., Ghantous, A., Keser, T., Madunić, J., Bošković, M., et al. (2019). Antagonistic and synergistic epigenetic modulation using orthologous CRISPR/dCas9-based modular system. Nucleic Acids Res. *47*, 9637–9657.

Jost, D., Rosa, A., Vaillant, C., and Everaers, R. (2018). 6 - a polymer physics view on universal and sequence-specific aspects of chromosome folding. In Nuclear Architecture and Dynamics, C. Lavelle and J.-M. Victor, eds. (Academic Press), pp. 149–169.

Jost, M., Santos, D.A., Saunders, R.A., Horlbeck, M.A., Hawkins, J.S., Scaria, S.M., Norman, T.M., Hussmann, J.A., Liem, C.R., Gross, C.A., and Weissman, J.S. (2020). Titrating gene expression using libraries of systematically attenuated CRISPR guide RNAs. Nat. Biotechnol. *38*, 355–364.

Joyce, E.F., Williams, B.R., Xie, T., and Wu, C.T. (2012). Identification of genes that promote or antagonize somatic homolog pairing using a high-throughput FISH-based screen. PLoS Genet. *8*, e1002667.

Kabadi, A.M., Thakore, P.I., Vockley, C.M., Ousterout, D.G., Gibson, T.M., Guilak, F., Reddy, T.E., and Gersbach, C.A. (2015). Enhanced MyoD-induced transdifferentiation to a myogenic lineage by fusion to a potent transactivation domain. ACS Synth. Biol. *4*, 689–699.

Keung, A.J., Bashor, C.J., Kiriakov, S., Collins, J.J., and Khalil, A.S. (2014). Using targeted chromatin regulators to engineer combinatorial and spatial transcriptional regulation. Cell *158*, 110–120.

Khalil, A.S., Lu, T.K., Bashor, C.J., Ramirez, C.L., Pyenson, N.C., Joung, J.K., and Collins, J.J. (2012). A synthetic biology framework for programming eukaryotic transcription functions. Cell *150*, 647–658.

Khanna, N., Zhang, Y., Lucas, J.S., Dudko, O.K., and Murre, C. (2019). Chromosome dynamics near the sol-gel phase transition dictate the timing of remote genomic interactions. Nat. Commun. *10*, 2771.

Khurana, E., Fu, Y., Chakravarty, D., Demichelis, F., Rubin, M.A., and Gerstein, M. (2016). Role of non-coding sequence variants in cancer. Nat. Rev. Genet. *17*, 93–108.

Kim, S., Beltran, B., Irnov, I., and Jacobs-Wagner, C. (2019a). Long-distance cooperative and antagonistic RNA polymerase dynamics via DNA supercoiling. Cell *179*, 106–119.e16.

Kim, J.H., Rege, M., Valeri, J., Dunagin, M.C., Metzger, A., Titus, K.R., Gilgenast, T.G., Gong, W., Beagan, J.A., Raj, A., and Phillips-Cremins, J.E. (2019b). LADL: light-activated dynamic looping for endogenous gene expression control. Nat. Methods *16*, 633–639.

Klattenhoff, C.A., Scheuermann, J.C., Surface, L.E., Bradley, R.K., Fields, P.A., Steinhauser, M.L., Ding, H., Butty, V.L., Torrey, L., Haas, S., et al. (2013). Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell *152*, 570–583.

Kraft, K., Geuer, S., Will, A.J., Chan, W.L., Paliou, C., Borschiwer, M., Harabula, I., Wittler, L., Franke, M., Ibrahim, D.M., et al. (2015). Deletions, inversions, duplications: engineering of structural variants using CRISPR/Cas in mice. Cell Rep. *10*, 833–839.

Kragesteen, B.K., Spielmann, M., Paliou, C., Heinrich, V., Schöpflin, R., Esposito, A., Annunziatella, C., Bianco, S., Chiariello, A.M., Jerković, I., et al. (2018). Dynamic 3D chromatin architecture contributes to enhancer specificity and limb morphogenesis. Nat. Genet. *50*, 1463–1473.

Krietenstein, N., Abraham, S., Venev, S.V., Abdennur, N., Gibcus, J., Hsieh, T.-H.S., Parsi, K.M., Yang, L., Maehr, R., Mirny, L.A., et al. (2020). Ultrastructural details of mammalian chromosome architecture. Mol. Cell *78*, 554–565.e7.

Lai, W.K.M., and Pugh, B.F. (2017). Understanding nucleosome dynamics and their links to gene expression and DNA replication. Nat. Rev. Mol. Cell Biol. *18*, 548–562.

Lakadamyali, M., and Cosma, M.P. (2020). Visualizing the genome in high resolution challenges our textbook understanding. Nat. Methods *17*, 371–379.

Lampo, T.J., Kennard, A.S., and Spakowitz, A.J. (2016). Physical modeling of dynamic coupling between chromosomal loci. Biophys. J. *110*, 338–347.

Le Gros, M.A., Clowney, E.J., Magklara, A., Yen, A., Markenscoff-Papadimitriou, E., Colquitt, B., Myllys, M., Kellis, M., Lomvardas, S., and Larabell, C.A. (2016). Soft X-ray tomography reveals gradual chromatin compaction and reorganization during neurogenesis in vivo. Cell Rep. *17*, 2125–2136.

Le, T.B., and Laub, M.T. (2016). Transcription rate and transcript length drive formation of chromosomal interaction domain boundaries. EMBO J. *35*, 1582–1595.

Le, T.T., Gao, X., Park, S.H., Lee, J., Inman, J.T., Lee, J.H., Killian, J.L., Badman, R.P., Berger, J.M., and Wang, M.D. (2019). Synergistic coordination of chromatin torsional mechanics and topoisomerase activity. Cell *179*, 619–631.e15.

Leidescher, S., Nübler, J., Feodorova, Y., Hildebrand, E., Ullrich, S., Bultmann, S., Link, S., Thanisch, K., Dekker, J., Leonhardt, H., et al. (2020). Spatial organization of transcribed eukaryotic genes. bioRxiv. https://doi.org/10.1101/2020.05.20.106591.

Lewandowski, J.P., Du, F., Zhang, S., Powell, M.B., Falkenstein, K.N., Ji, H., and Vokes, S.A. (2015). Spatiotemporal regulation of GLI target genes in the mammalian limb bud. Dev. Biol. *406*, 92–103.

Li, G., Cai, L., Chang, H., Hong, P., Zhou, Q., Kulakova, E.V., Kolchanov, N.A., and Ruan, Y. (2014). Chromatin Interaction Analysis with Paired-End Tag (ChIA-PET) sequencing technology and application. BMC Genomics *15*, https://doi.org/10.1186/1471-2164-15-S12-S11.

Liao, W., Liu, B., Chang, C.C., Lin, L.J., Lin, C., Chen, B.S., and Xie, Z. (2018). Functional characterization of insulation effect for synthetic gene circuits in mammalian cells. ACS Synth. Biol. *7*, 412–418.

Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science *326*, 289–293.

Liu, P., Chen, M., Liu, Y., Qi, L.S., and Ding, S. (2018). CRISPR-based chromatin remodeling of the endogenous Oct4 or Sox2 locus enables reprogramming to pluripotency. Cell Stem Cell *22*, 252–261.e4.

Liu, X.S., Wu, H., Ji, X., Stelzer, Y., Wu, X., Czauderna, S., Shu, J., Dadon, D., Young, R.A., and Jaenisch, R. (2016). Editing DNA methylation in the mammalian genome. Cell *167*, 233–247.e17.

Lu, L., Liu, X., Huang, W.K., Giusti-Rodríguez, P., Cui, J., Zhang, S., Xu, W., Wen, Z., Ma, S., Rosen, J.D., et al. (2020). Robust Hi-C maps of enhancer-promoter interactions reveal the function of non-coding genome in neural development and diseases. Mol. Cell *79*, 521–534.e15.

Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature *389*, 251–260.

Ma, J., and Wang, M.D. (2016). DNA supercoiling during transcription. Biophys. Rev. *8*, 75–87.

Maeshima, K., Ide, S., and Babokhov, M. (2019). Dynamic chromatin organization without the 30-nm fiber. Curr. Opin. Cell Biol. *58*, 95–104.

Marinov, G.K., Trevino, A.E., Xiang, T., Kundaje, A., Grossman, A.R., and Greenleaf, W.J. (2020). Transcription-dependent domain-scale 3D genome organization in dinoflagellates. bioRxiv. https://doi.org/10.1101/2020.07.01.181685.

Marko, J.F. (2018). 1.DNA mechanics. In Nuclear Architecture and Dynamics, C. Lavelle and J.-M. Victor, eds. (Academic Press), pp. 3–40.

Marx, N., Grünwald-Gruber, C., Bydlinski, N., Dhiman, H., Ngoc Nguyen, L., Klanert, G., and Borth, N. (2018). CRISPR-based targeted epigenetic editing enables gene expression modulation of the silenced beta-galactoside alpha-2,6-sialyltransferase 1 in CHO cells. Biotechnol. J. *13*, e1700217.

Mateo, L.J., Murphy, S.E., Hafner, A., Cinquini, I.S., Walker, C.A., and Boettiger, A.N. (2019). Visualizing DNA folding and RNA in embryos at single-cell resolution. Nature *568*, 49–54.

Milo, R., and Phillips, R. (2015). Cell Biology by the Numbers (Garland Science).

Mirny, L.A., Imakaev, M., and Abdennur, N. (2019). Two major mechanisms of chromosome organization. Curr. Opin. Cell Biol. *58*, 142–152.

Miyanari, Y., Ziegler-Birling, C., and Torres-Padilla, M.E. (2013). Live visualization of chromatin dynamics with fluorescent TALEs. Nat. Struct. Mol. Biol. *20*, 1321–1324.

Molina, O., Vargiu, G., Abad, M.A., Zhiteneva, A., Jeyaprakash, A.A., Masumoto, H., Kouprina, N., Larionov, V., and Earnshaw, W.C. (2016). Epigenetic engineering reveals a balance between histone modifications and transcription in kinetochore maintenance. Nat. Commun. *7*, 13334.

Moller, J., and de Pablo, J.J. (2020). Bottom-up meets top-down: the crossroads of multiscale chromatin modeling. Biophys. J. *118*, 2057–2065.

Morgan, S.L., Mariano, N.C., Bermudez, A., Arruda, N.L., Wu, F., Luo, Y., Shankar, G., Jia, L., Chen, H., Hu, J.-F., et al. (2017). Manipulation of nuclear architecture through CRISPR-mediated chromosomal looping. Nat. Commun. *8*, 15993.

Morillon, A., Karabetsou, N., Nair, A., and Mellor, J. (2005). Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. Mol. Cell *18*, 723–734.

Mumbach, M.R., Rubin, A.J., Flynn, R.A., Dai, C., Khavari, P.A., Greenleaf, W.J., and Chang, H.Y. (2016). HiChIP: efficient and sensitive analysis of protein-directed genome architecture. Nat. Methods *13*, 919–922.

Naughton, C., Avlonitis, N., Corless, S., Prendergast, J.G., Mati, I.K., Eijk, P.P., Cockroft, S.L., Bradley, M., Ylstra, B., and Gilbert, N. (2013). Transcription forms and remodels supercoiling domains unfolding large-scale chromatin structures. Nat. Struct. Mol. Biol. *20*, 387–395.

Nickolls, A.R., Lee, M.M., Espinoza, D.F., Szczot, M., Lam, R.M., Wang, Q., Beers, J., Zou, J., Nguyen, M.Q., Solinski, H.J., et al. (2020). Transcriptional programming of human mechanosensory neuron subtypes from pluripotent stem cells. Cell Rep. *30*, 932–946.e7.

Nir, G., Farabella, I., Estrada, C.P., Ebeling, C.G., Beliveau, B.J., Sasaki, H.M., Lee, S.D., Nguyen, S.C., McCole, R.B., Chattoraj, S., et al. (2018). Walking along chromosomes with super-resolution imaging, contact maps, and integrative modeling. PLoS Genet. *14*, e1007872.

Nora, E.P., Goloborodko, A., Valton, A.L., Gibcus, J.H., Uebersohn, A., Abdennur, N., Dekker, J., Mirny, L.A., and Bruneau, B.G. (2017). Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. Cell *169*, 930–944.e22.

Nora, E.P., Lajoie, B.R., Schulz, E.G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., van Berkum, N.L., Meisig, J., Sedat, J., et al. (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature *485*, 381–385.

Nozawa, R.S., and Gilbert, N. (2019). RNA: nuclear glue for folding the genome. Trends Cell Biol. *29*, 201–211.

Nuebler, J., Fudenberg, G., Imakaev, M., Abdennur, N., and Mirny, L.A. (2018). Chromatin organization by an interplay of loop extrusion and compartmental segregation. Proc. Natl. Acad. Sci. USA *115*, E6697–E6706.

Ou, H.D., Phan, S., Deerinck, T.J., Thor, A., Ellisman, M.H., and O'Shea, C.C. (2017). ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. Science *357*, eaag0025.

Oudelaar, A.M., Beagrie, R.A., Gosden, M., de Ornellas, S., Georgiades, E., Kerry, J., Hidalgo, D., Carrelha, J., Shivalingam, A., El-Sagheer, A.H., et al. (2020). Dynamics of the 4D genome during in vivo lineage specification and differentiation. Nat. Commun. *11*, 2722.

Pang, B., and Snyder, M.P. (2020). Systematic identification of silencers in human cells. Nat. Genet. *52*, 254–263.

Park, J.W., Lee, J.K., Sheu, K.M., Wang, L., Balanis, N.G., Nguyen, K., Smith, B.A., Cheng, C., Tsai, B.L., Cheng, D., et al. (2018). Reprogramming normal human epithelial tissues to a common, lethal neuroendocrine cancer lineage. Science *362*, 91–95.

Park, M., Patel, N., Keung, A.J., and Khalil, A.S. (2019). Engineering epigenetic regulation using synthetic read-write modules. Cell *176*, 227–238.e20.

Paulsen, J., Sekelja, M., Oldenburg, A.R., Barateau, A., Briand, N., Delbarre, E., Shah, A., Sørensen, A.L., Vigouroux, C., Buendia, B., and Collas, P. (2017). Chrom3D: three-dimensional genome modeling from Hi-C and nuclear lamin-genome contacts. Genome Biol. *18*, 21.

Pausch, P., Al-Shayeb, B., Bisom-Rapp, E., Tsuchida, C.A., Li, Z., Cress, B.F., Knott, G.J., Jacobsen, S.E., Banfield, J.F., and Doudna, J.A. (2020). CRISPR-CasΦ from huge phages is a hypercompact genome editor. Science *369*, 333–337.

Pennacchio, L.A., Bickmore, W., Dean, A., Nobrega, M.A., and Bejerano, G. (2013). Enhancers: five essential questions. Nat. Rev. Genet. *14*, 288–295.

Phillips, R., Kondev, J., Theriot, J., and Garcia, H. (2012). Physical Biology of the Cell (Garland Science).

Phillips-Cremins, J.E., Sauria, M.E.G., Sanyal, A., Gerasimova, T.I., Lajoie, B.R., Bell, J.S.K., Ong, C.-T., Hookway, T.A., Guo, C., Sun, Y., et al. (2013). Architectural protein subclasses shape 3-D organization of genomes during lineage commitment. Cell *153*, 1281–1295.

Polovnikov, K.E., Gherardi, M., Cosentino-Lagomarsino, M., and Tamm, M.V. (2018). Fractal folding and medium viscoelasticity contribute jointly to chromosome dynamics. Phys. Rev. Lett. *120*, 088101.

Puc, J., Kozbial, P., Li, W., Tan, Y., Liu, Z., Suter, T., Ohgi, K.A., Zhang, J., Aggarwal, A.K., and Rosenfeld, M.G. (2015). Ligand-dependent enhancer activation regulated by topoisomerase-I activity. Cell *160*, 367–380.

Quinodoz, S.A., Bhat, P., Ollikainen, N., Jachowicz, J.W., Banerjee, A.K., Chovanec, P., Blanco, M.R., Chow, A., Markaki, Y., Plath, K., and Guttman, M. (2020). RNA promotes the formation of spatial compartments in the nucleus. bioRxiv. https://doi.org/10.1101/2020.08.25.267435.

Quinodoz, S.A., Ollikainen, N., Tabak, B., Palla, A., Schmidt, J.M., Detmar, E., Lai, M.M., Shishkin, A.A., Bhat, P., and Takei, Y. (2018). Higher-order inter-chromosomal hubs shape 3D genome organization in the nucleus. Cell *174*, 744–757.e24.

Raj, A., van den Bogaard, P., Rifkin, S.A., van Oudenaarden, A., and Tyagi, S. (2008). Imaging individual mRNA molecules using multiple singly labeled probes. Nat. Methods *5*, 877–879.

Rao, S.S.P., Huang, S.-C., Hilaire, G.St., Engreitz, B., Perez, J.M., Kieffer-Kwon, E.M., Sanborn, K.-R., Johnstone, A.L., Bascom, S.E., Bochkov, G.D., et al. (2017). Cohesin loss eliminates all loop domains. Cell *171*, 305–320.e24.

Rao, S.S.P., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T., Sanborn, A.L., Machol, I., Omer, A.D., Lander, E.S., and Aiden, E.L. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell *159*, 1665–1680.

Ricci, M.A., Manzo, C., García-Parajo, M.F., Lakadamyali, M., and Cosma, M.P. (2015). Chromatin fibers are formed by heterogeneous groups of nucleosomes in vivo. Cell *160*, 1145–1158.

Rivera-Mulia, J.C., Kim, S., Gabr, H., Chakraborty, A., Ay, F., Kahveci, T., and Gilbert, D.M. (2019). Replication timing networks reveal a link between transcription regulatory circuits and replication timing control. Genome Res. *29*, 1415–1428.

Rowley, M.J., and Corces, V.G. (2018). Organizational principles of 3D genome architecture. Nat. Rev. Genet. *19*, 789–800.

Rowley, M.J., Nichols, M.H., Lyu, X., Ando-Kuri, M., Rivera, I.S.M., Hermetz, K., Wang, P., Ruan, Y., and Corces, V.G. (2017). Evolutionarily conserved principles predict 3D chromatin organization. Mol. Cell *67*, 837–852.e7.

Sakabe, N.J., Savic, D., and Nobrega, M.A. (2012). Transcriptional enhancers in development and disease. Genome Biol. *13*, 238.

Saldaña-Meyer, R., Rodriguez-Hernaez, J., Escobar, T., Nishana, M., Jácome-López, K., Nora, E.P., Bruneau, B.G., Tsirigos, A., Furlan-Magaril, M., Skok, J., and Reinberg, D. (2019). RNA interactions are essential for CTCF-mediated genome organization. Mol. Cell *76*, 412–422.e5.

Sanborn, A.L., Rao, S.S.P., Huang, S.C., Durand, N.C., Huntley, M.H., Jewett, A.I., Bochkov, I.D., Chinnappan, D., Cutkosky, A., Li, J., et al. (2015). Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. Proc. Natl. Acad. Sci. USA *112*, E6456–E6465.

Schoenfelder, S., and Fraser, P. (2019). Long-range enhancer–promoter contacts in gene expression control. Nat. Rev. Genet. *20*, 437–455.

Schuijers, J., Manteiga, J.C., Weintraub, A.S., Day, D.S., Zamudio, A.V., Hnisz, D., Lee, T.I., and Young, R.A. (2018). Transcriptional dysregulation of MYC reveals common enhancer-docking mechanism. Cell Rep. *23*, 349–360.

Schwarzer, W., Abdennur, N., Goloborodko, A., Pekowska, A., Fudenberg, G., Loe-Mie, Y., Fonseca, N.A., Huber, W., Haering, C.H., Mirny, L., and Spit, F. (2017). Two independent modes of chromatin organization revealed by cohesin removal. Nature *551*, 51–56.

Seisenberger, S., Peat, J.R., Hore, T.A., Santos, F., Dean, W., and Reik, W. (2013). Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. Philos. Trans. R. Soc. Lond. B Biol. Sci. *368*, 20110330.

Seki, Y., Hayashi, K., Itoh, K., Mizugaki, M., Saitou, M., and Matsui, Y. (2005). Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev. Biol. *278*, 440–458.

Serra, F., Baù, D., Goodstadt, M., Castillo, D., Filion, G.J., and Marti-Renom, M.A. (2017). Automatic analysis and 3D-modelling of Hi-C data using TADbit reveals structural features of the fly chromatin colors. PLoS Comput. Biol. *13*, e1005665.

Sevier, S.A. (2020). Mechanical properties of DNA replication. Phys. Rev. Res. *2*, 023280.

Sevier, S.A., and Levine, H. (2018). Properties of gene expression and chromatin structure with mechanically regulated elongation. Nucleic Acids Res. *46*, 5924–5934.

Shaban, H.A., Barth, R., and Bystricky, K. (2018). Formation of correlated chromatin domains at nanoscale dynamic resolution during transcription. Nucleic Acids Res. *46*, e77.

Shaban, H.A., Barth, R., Recoules, L., and Bystricky, K. (2020). Hi-D: nanoscale mapping of nuclear dynamics in single living cells. Genome Biol. *21*, 95.

Sharma, S.V., Lee, D.Y., Li, B., Quinlan, M.P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M.A., et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell *141*, 69–80.

Sheth, R., Barozzi, I., Langlais, D., Osterwalder, M., Nemec, S., Carlson, H.L., Stadler, H.S., Visel, A., Drouin, J., and Kmita, M. (2016). Distal limb patterning requires modulation of cis-regulatory activities by HOX13. Cell Rep. *17*, 2913–2926.

Shin, Y., Berry, J., Pannucci, N., Haataja, M.P., Toettcher, J.E., and Brangwynne, C.P. (2017). Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. Cell *168*, 159–171.e14.

Shin, Y., Chang, Y.-C., Lee, D.S.W., Berry, J., Sanders, D.W., Ronceray, P., Wingreen, N.S., Haataja, M., and Brangwynne, C.P. (2018). Liquid nuclear condensates mechanically sense and restructure the genome. Cell *175*, 1481–1491.e13.

Shrinivas, K., Sabari, B.R., Coffey, E.L., Klein, I.A., Boija, A., Zamudio, A.V., Schuijers, J., Hannett, N.M., Sharp, P.A., Young, R.A., and Chakraborty, A.K. (2019). Enhancer features that drive formation of transcriptional condensates. Mol. Cell *75*, 549–561.e7.

Smith, E.A., McDermott, G., Do, M., Leung, K., Panning, B., Le Gros, M.A., and Larabell, C.A. (2014). Quantitatively imaging chromosomes by correlated cryo-fluorescence and soft X-ray tomographies. Biophys. J. *107*, 1988–1996.

Spivakov, M., and Fisher, A.G. (2007). Epigenetic signatures of stem-cell identity. Nat. Rev. Genet. *8*, 263–271.

Stadhouders, R., Vidal, E., Serra, F., Di Stefano, B., Le Dily, F., Quilez, J., Gomez, A., Collombet, S., Berenguer, C., Cuartero, Y., et al. (2018). Transcription factors orchestrate dynamic interplay between genome topology and gene regulation during cell reprogramming. Nat. Genet. *50*, 238–249.

Stasevich, T.J., Hayashi-Takanaka, Y., Sato, Y., Maehara, K., Ohkawa, Y., Sakata-Sogawa, K., Tokunaga, M., Nagase, T., Nozaki, N., McNally, J.G., and Kimura, H. (2014). Regulation of RNA polymerase II activation by histone acetylation in single living cells. Nature *516*, 272–275.

Stevens, T.J., Lando, D., Basu, S., Atkinson, L.P., Cao, Y., Lee, S.F., Leeb, M., Wohlfahrt, K.J., Boucher, W., O'Shaughnessy-Kirwan, A., et al. (2017). 3D structures of individual mammalian genomes studied by single-cell Hi-C. Nature *544*, 59–64.

Stik, G., Vidal, E., Barrero, M., Cuartero, S., Vila-Casadesús, M., Mendieta-Esteban, J., Tian, T.V., Choi, J., Berenguer, C., Abad, A., et al. (2020). CTCF is dispensable for immune cell transdifferentiation but facilitates an acute inflammatory response. Nat. Genet. *52*, 655–661.

Straight, A.F., Belmont, A.S., Robinett, C.C., and Murray, A.W. (1996). GFP tagging of budding yeast chromosomes reveals that protein–protein interactions can mediate sister chromatid cohesion. Curr. Biol. *6*, 1599–1608.

Su, J.H., Zheng, P., Kinrot, S.S., Bintu, B., and Zhuang, X. (2020). Genome-scale imaging of the 3D organization and transcriptional activity of chromatin. Cell *182*, 1641–1659.e26.

Symmons, O., Uslu, V.V., Tsujimura, T., Ruf, S., Nassari, S., Schwarzer, W., Ettwiller, L., and Spitz, F. (2014). Functional and topological characteristics of mammalian regulatory domains. Genome Res. *24*, 390–400.

Tan, L., Xing, D., Chang, C.H., Li, H., and Xie, X.S. (2018). Three-dimensional genome structures of single diploid human cells. Science *361*, 924–928.

Tanenbaum, M.E., Gilbert, L.A., Qi, L.S., Weissman, J.S., and Vale, R.D. (2014). A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell *159*, 635–646.

Tasan, I., Sustackova, G., Zhang, L., Kim, J., Sivaguru, M., HamediRad, M., Wang, Y., Genova, J., Ma, J., Belmont, A.S., and Zhao, H. (2018). CRISPR/Cas9-mediated knock-in of an optimized TetO repeat for live cell imaging of endogenous loci. Nucleic Acids Res. *46*, e100.

Tekel, S.J., Vargas, D.A., Song, L., LaBaer, J., Caplan, M.R., and Haynes, K.A. (2018). Tandem histone-binding domains enhance the activity of a synthetic chromatin effector. ACS Synth. Biol. *7*, 842–852.

Teves, S.S., and Henikoff, S. (2014). Transcription-generated torsional stress destabilizes nucleosomes. Nat. Struct. Mol. Biol. *21*, 88–94.

Tsubouchi, T., Soza-Ried, J., Brown, K., Piccolo, F.M., Cantone, I., Landeira, D., Bagci, H., Hochegger, H., Merkenschlager, M., and Fisher, A.G. (2013). DNA synthesis is required for reprogramming mediated by stem cell fusion. Cell *152*, 873–883.

Van, M.V., Fujimori, T., and Bintu, L. (2020). Nanobody-mediated control of gene expression and epigenetic memory. Synth. Biol. https://doi.org/10.1101/2020.09.09.290015.

Veerapandian, V., Ackermann, J.O., Srivastava, Y., Malik, V., Weng, M., Yang, X., and Jauch, R. (2018). Directed evolution of reprogramming factors by cell selection and sequencing. Stem Cell Reports *11*, 593–606.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Südhof, T.C., and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. Nature *463*, 1035–1041.

Villaseñor, R., Pfaendler, R., Ambrosi, C., Butz, S., Giuliani, S., Bryan, E., Sheahan, T.W., Gable, A.L., Schmolka, N., Manzo, M., et al. (2020). ChromID identifies the protein interactome at chromatin marks. Nat. Biotechnol. *38*, 728–736.

Wang, C., Ward, M.E., Chen, R., Liu, K., Tracy, T.E., Chen, X., Xie, M., Sohn, P.D., Ludwig, C., Meyer-Franke, A., et al. (2017). Scalable production of iPSC-derived human neurons to identify tau-lowering compounds by high-content screening. Stem Cell Reports *9*, 1221–1233.

Wang, H., Xu, X., Nguyen, C.M., Liu, Y., Gao, Y., Lin, X., Daley, T., Kipniss, N.H., La Russa, M., and Qi, L.S. (2018). CRISPR-mediated programmable 3D genome positioning and nuclear organization. Cell *175*, 1405–1417.e14.

Wang, N.B., Beitz, A.M., and Galloway, K.E. (2020). Engineering cell fate: Applying synthetic biology to cellular reprogramming. Current Opinion in Systems Biology, In press. https://doi.org/10.1016/j.coisb.2020.09.002.

Wang, Z., Chivu, A.G., Choate, L.A., Rice, E.J., Miller, D.C., Chu, T., Chou, S.-P., Kingsley, N.B., Peterson, J.L., Finno, C.J., et al. (2020a). Accurate imputation of histone modifications using transcription. Genomics.

Wang, J., Jiang, X., Zhao, L., Zuo, S., Chen, X., Zhang, L., Lin, Z., Zhao, X., Qin, Y., Zhou, X., and Yu, X.-Y. (2020b). Lineage reprogramming of fibroblasts into induced cardiac progenitor cells by CRISPR/Cas9-based transcriptional activators. Acta Pharm. Sin. B *10*, 313–326.

Wapinski, O.L., Vierbuchen, T., Qu, K., Lee, Q.Y., Chanda, S., Fuentes, D.R., Giresi, P.G., Ng, Y.H., Marro, S., Neff, N.F., et al. (2013). Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. Cell *155*, 621–635.

Yang, J., and Corces, V.G. (2011). Chromatin insulators: a role in nuclear organization and gene expression. Adv. Cancer Res. *110*, 43–76.

Yeung, E., Dy, A.J., Martin, K.B., Ng, A.H., Del Vecchio, D., Beck, J.L., Collins, J.J., and Murray, R.M. (2017). Biophysical constraints arising from compositional context in synthetic gene networks. Cell Syst. *5*, 11–24.e12.

Yokoshi, M., Segawa, K., and Fukaya, T. (2020). Visualizing the role of boundary elements in enhancer-promoter communication. Mol. Cell *78*, 224–235.e5.

Zamudio, A.V., Dall'Agnese, A., Henninger, J.E., Manteiga, J.C., Afeyan, L.K., Hannett, N.M., Coffey, E.L., Li, C.H., Oksuz, O., Sabari, B.R., et al. (2019). Mediator condensates localize signaling factors to key cell identity genes. Mol. Cell *76*, 753–766.e6.

Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., Volz, S.E., Joung, J., van der Oost, J., Regev, A., et al. (2015). Cpf1 is a single RNA-guided endonuclease of a Class 2 CRISPR-cas system. Cell *163*, 759–771.

Zhang, H., Emerson, D.J., Gilgenast, T.G., Titus, K.R., Lan, Y., Huang, P., Zhang, D., Wang, H., Keller, C.A., Giardine, B., et al. (2019). Chromatin structure dynamics during the mitosis-to-G1 phase transition. Nature *576*, 158–162.

Zhao, X., Sternsdorf, T., Bolger, T.A., Evans, R.M., and Yao, T.P. (2005). Regulation of MEF2 by histone deacetylase 4- and SIRT1 deacetylase-mediated lysine modifications. Mol. Cell. Biol. *25*, 8456–8464.

Zhao, Z., Tavoosidana, G., Sjölinder, M., Göndör, A., Mariano, P., Wang, S., Kanduri, C., Lezcano, M., Sandhu, K.S., Singh, U., et al. (2006). Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat. Genet. *38*, 1341–1347.

Zhu, J.J., and Cheng, A.W. (2020). JACKIE: fast enumeration of single- and multi-copy CRISPR binding sites. bioRxiv. https://doi.org/10.1101/2020.02.27.968933.

Zuiddam, M., Everaers, R., and Schiessel, H. (2017). Physics behind the mechanical nucleosome positioning code. Phys. Rev. E *96*, 052412.