REVIEW

https://doi.org/10.1038/s41586-019-1182-7

Transcription factors and 3D genome conformation in cell–fate decisions

Ralph Stadhouders[1,2]*, Guillaume J. Filion[3,4] & Thomas Graf[3,4]*

How cells adopt different identities has long fascinated biologists. Signal transduction in response to environmental cues results in the activation of transcription factors that determine the gene–expression program characteristic of each cell type. Technological advances in the study of 3D chromatin folding are bringing the role of genome conformation in transcriptional regulation to the fore. Characterizing this role of genome architecture has profound implications, not only for differentiation and development but also for diseases including developmental malformations and cancer. Here we review recent studies indicating that the interplay between transcription and genome conformation is a driving force for cell–fate decisions.

The capacity of cells to acquire new fates is central to the development of multicellular organisms. A specific cellular state, which is defined by the cell's phenotype and functional characteristics, ultimately represents the readout of a specific gene-expression program[1]. Starting with the division of a fertilized egg, a complex cascade of successive cell-state transitions results in the formation of hundreds of different cell types. Cell-fate decisions are driven by changes in environmental cues, such as cell–cell interactions, cytokines and other soluble factors that trigger signal transduction into the nucleus. Signalling pathways converge in the activation or silencing of DNA sequence-specific regulators, most prominently transcription factors, which comprise around 8% of all human genes[2,3]. Transcription factors act by binding to specific DNA motifs within gene regulatory elements, that is, promoters and enhancers[4]. Whereas promoters always localize directly adjacent to the transcription start sites of genes, enhancers often reside at a considerable distance from their target genes[5]. Once bound, transcription factors either promote or impede recruitment of the cellular machineries necessary for gene transcription into mRNA, for chromatin remodelling or for histone and DNA modifications. In turn, chromatin modifications constrain the access of transcription factors to regulatory elements. Different transcription factors may exhibit different activities. Whereas some are ubiquitously expressed, others show a more lineage-restricted expression pattern and many have the ability to bind to otherwise inaccessible nucleosomal DNA and act as 'pioneer' transcription factors[3,6]. The establishment and maintenance of cell-type-specific gene-expression programs therefore results from the interaction between transcription factors and the chromatin landscape that they encounter[7,8].

Genome structure has long been approached as a one-dimensional phenomenon (that is, as a linear fibre with functional elements separated by a certain distance), but several decades of research have shown that chromatin adopts a complex three-dimensional conformation within the nucleus. This insight has functional implications for almost all nuclear processes, including transcriptional regulation[9,10]. For example, spatial folding of chromatin provides a mechanism for distal enhancers to connect with their target promoters[11]. Therefore, genome conformation is an integral part of the chromatin landscape that transcription factors must navigate to exert their gene regulatory functions.

The various components that orchestrate transcriptional regulation act in a complex, multilayered and interconnected fashion. Therefore, a seemingly minor event, such as the activation of a single transcription factor, can have enormous consequences and induce a change of cell fate. The concept of 'emergent properties' has been defined as characteristics that appear from the interaction between components at various levels of organization, which go beyond those that can be predicted from studying the individual components[12]. On the basis of this definition, cell identity can be considered as an emergent property that arises from the interplay between transcription factors, chromatin-associated proteins, epigenetic modifications and a spatially organized genome (Fig. 1). However, cell-fate-instructive transcription factors stand out among these components, in that they possess the ability to short-circuit signal transduction processes when overexpressed. This often results in the complete rewiring of a cell's gene-expression program and the reprogramming of one cell type into another[13]. Moreover, these transcription factors are also required for maintaining a differentiated cellular identity, as removing them can induce de-differentiation and re-specification into alternative lineages[13]. Hence, transcription factors can be considered both as catalysts and as agents required for the emergent property of cell identity. In this scenario, transcription factors would interact with a three-dimensionally organized chromatin landscape and its associated components (including various proteins and non-coding RNAs) during differentiation, leading to a self-organized transition to a new stable state. This process results in the fine-tuning and ultimately in the consolidation of the cell's new transcriptome, manifested as a change of cell fate.

Here we review mechanisms that shape 3D genome conformation and discuss recent studies that address the role of genome conformation in the acquisition of new cell identities from the perspective of mechanisms driven by transcription factors. We focus on how transcription factors orchestrate the dynamic interplay between genome form and function, and propose several distinct roles that genome conformation may have in the context of gene regulation and cell-fate transitions.

Basic principles of 3D genome folding

The past decade has brought tremendous progress in our understanding of the spatial nature of the chromatin landscape that influences the action of transcription factors[14,15]. Powerful technologies, including super-resolution microscopy and chromosome conformation capture, have provided a detailed and multilevel view of how eukaryotic genomes are organized in the nucleus. Moreover, genome editing and

[1]Department of Pulmonary Medicine, Erasmus MC, Rotterdam, the Netherlands. [2]Department of Cell Biology, Erasmus MC, Rotterdam, the Netherlands. [3]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain. [4]Universitat Pompeu Fabra (UPF), Barcelona, Spain. *e-mail: r.stadhouders@erasmusmc.nl; thomas.graf@crg.eu

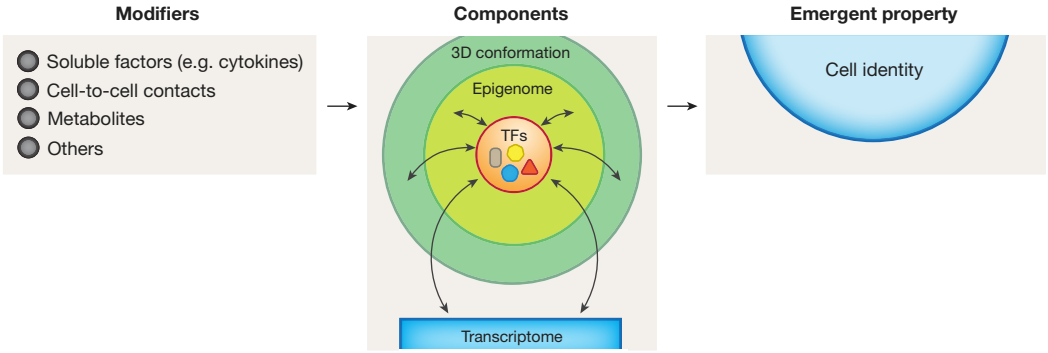

Fig. 1 | Cell identity as a transcription-factor-driven emergent property. Signal transduction induced by external cues (or modifiers, for example, cytokines and other soluble factors, cell–cell contacts or metabolites) modulates the activity of transcription factors (TFs). This process can be short-circuited by the forced expression of specific cell-fate-instructive transcription factors. Activated transcription factors, through their sequence-specific DNA-binding capacity, in turn interact with a chromatin landscape (epigenome) that adopts a specific 3D conformation within the nucleus. Through the recruitment of epigenetic modifiers and the transcriptional machinery, transcription factors modulate the cell's gene-expression program (transcriptome). Ultimately, the interplay between these nuclear components, orchestrated by transcription factors, results in the adoption of a specific cellular identity defined here as an emergent property.

near-instant protein depletion techniques, including degron-mediated proteolysis, have shed light on the underlying mechanisms. Below we discuss the current view on the folding principles of the genome (Fig. 2) and the possible role of transcription factors in shaping them.

Distinct and overlapping chromosome territories

As early as 1885, Carl Rabl described chromosome decondensation on exit of mitosis leading to the formation of confined nuclear territories for individual chromosomes[16]. The forces that create and maintain these territories remain poorly understood; however, attachment to the nuclear envelope appears to be important[17]. Although different chromosomes localize to distinct territories, regions of overlap do occur[18]. Selective separation of territories organizes chromosomes into two interchromosomal contact hubs: gene-dense segments of active (euchromatic) chromatin associated with RNA polymerase II (RNAPII) clustering around nuclear speckles, and RNAPII-depleted inactive (heterochromatic) chromatin enriched for ribosomal RNA genes and centromeric chromatin residing near the nucleolus[19]. Intermingling of chromosome territories has been proposed to be functionally important for gene regulation in various contexts and several transcription factors have been implicated in mediating these interchromosomal interactions[20–24]. However, functional interchromosomal communication between regulatory elements appears to be rare. An example is olfactory receptor gene choice in neurons, which is guided by a specific interchromosomal clustering of enhancers mediated by the LIM homeobox 2 (LHX2) transcription factor[23]. Moreover, CCCTC-binding factor (CTCF) was shown to tether chromatin regions to the nucleolus via interactions with nucleophosmin[25], indicating that transcription factors are capable of inducing chromatin repositioning to specific nuclear landmarks[26].

Chromosomal compartmentalization

The development of technologies to systematically interrogate interaction frequencies between genomic regions[27] has resulted in the discovery of several principles of 3D genome organization. Such genome-wide chromosome conformation capture (Hi-C) experiments reveal that each territory spatially segregates into two chromosome compartments predominantly consisting of either euchromatic (the A compartment) or heterochromatic (the B compartment) genome segments[27]. Whereas the A compartment occupies the nuclear interior, the B compartment resides near the nucleolus and the nuclear lamina[28–30]. Similar to the interaction interfaces between chromosome territories described above, intra-chromosomal genome conformation therefore predicts the biochemical activity of chromatin.

Functional studies of single gene loci have shown that chromatin-associated proteins, including transcription factors and chromatin-modifying enzymes, can induce nuclear repositioning and A–B compartment switching, irrespective of transcriptional changes[30–32]. For example, the transcription factors yin yang 1 (YY1) and CTCF have been implicated in tethering gene loci to the nuclear lamina or the nucleolus[25,33]. Of note, recruitment of a DNA-binding domain fused to a viral transactivator is sufficient to induce chromatin remodelling and gene repositioning towards the nuclear interior[31]. Moreover, in a study of B-cell reprogramming into induced pluripotent stem cells by the sequential expression of CCAAT/enhancer-binding protein alpha (C/EBPα) and the four Yamanaka factors—OCT4, SOX2, KLF4 and MYC (OSKM)—transcription-factor binding often predicted subsequent A–B compartment switching[34]. These experiments therefore suggest that the transcription-factor-driven dynamic behaviour of the chromatin landscape fuels A–B compartmentalization and gene positioning in the nucleus.

The process of phase separation has recently been proposed to have a prominent role in 3D genome organization[35,36]. Phase separation describes a phenomenon in which proteins self-organize into liquid-like droplets (or condensates), acting as membrane-less organelles that concentrate specific molecules and excluding others[37,38] (Fig. 3a). In such a process, sequence-specific transcription factors containing low-complexity disordered protein regions can form highly dynamic nuclear clusters that interact with the transcriptional co-activator Mediator[39] or RNAPII[40]. These findings are consistent with experiments that revealed spatial co-localization of binding sites for transcription factors or polycomb proteins in the nucleus[23,24,41–43]. Nuclear hub formation by factors that associate with either euchromatin (such as RNAPII[44], cyclin T1[45], Mediator[46], bromodomain-containing protein 4 (BRD4)[47] or transcription factors[39,40]) or heterochromatin (such as polycomb[48] and heterochromatin protein 1 (HP1α)[49,50]) thus provides a plausible mechanism for intra- and interchromosomal compartmentalization.

Intrachromosomal topological domains

Hi-C analyses revealed the presence of spatially insulated genomic regions commonly referred to as topologically associating domains (TADs)[51–53]. How these interaction domains are formed has been the topic of intense investigation. CTCF and the cohesin complex are enriched at TAD boundaries, which suggests that TADs form predominantly between CTCF–cohesin binding sites[52,54,55]. These and other observations have led to the loop extrusion model to explain how TADs are formed[56–58] (Fig. 3a). In this model, an extrusion factor (for example, the ring-shaped cohesin complex) begins to extrude a chromatin loop, much like threading yarn through the eye of a needle, until it encounters an extrusion barrier associated with the DNA. CTCF functions as a barrier for extrusion by interacting with cohesin only

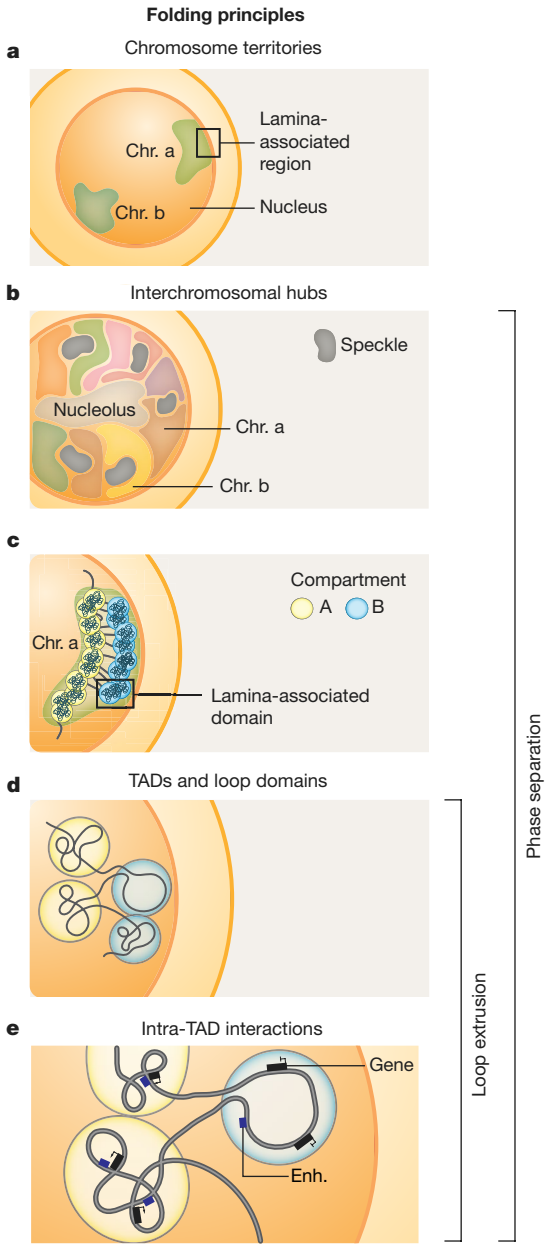

Fig. 2 | Three-dimensional folding principles of chromatin. a–**e**, Different chromatin folding principles within the nucleus are ordered from top to bottom according to their dimensions. Individual chromosomes (represented by chr. a and chr. b) occupy distinct territories (**a**) that form interchromosomal hubs, in which active chromatin resides near nuclear speckles and inactive chromatin is clustered around the nucleolus (**b**). **c**, Within chromosome territories, active and inactive chromatin segregate into A and B compartments, respectively. A-compartment domains (yellow spheres) are positioned towards the nuclear interior, whereas B-compartment domains (blue spheres) are enriched at the nuclear lamina, forming lamina-associated domains (LADs). **d**, At smaller scales, chromatin is organized into insulated spatial neighbourhoods referred to as TADs or loop domains. **e**, Within these domains, gene expression is controlled by regulatory elements involving dynamic interactions between promoters and enhancers that are relatively restrained by the boundaries of the TAD or loop domain in which they reside. Phase-separation processes potentially act at each level of organization, whereas in mammals, loop extrusion has been specifically implicated in the formation of TADs, loop domains and promoter–enhancer interactions. Enh., enhancer.

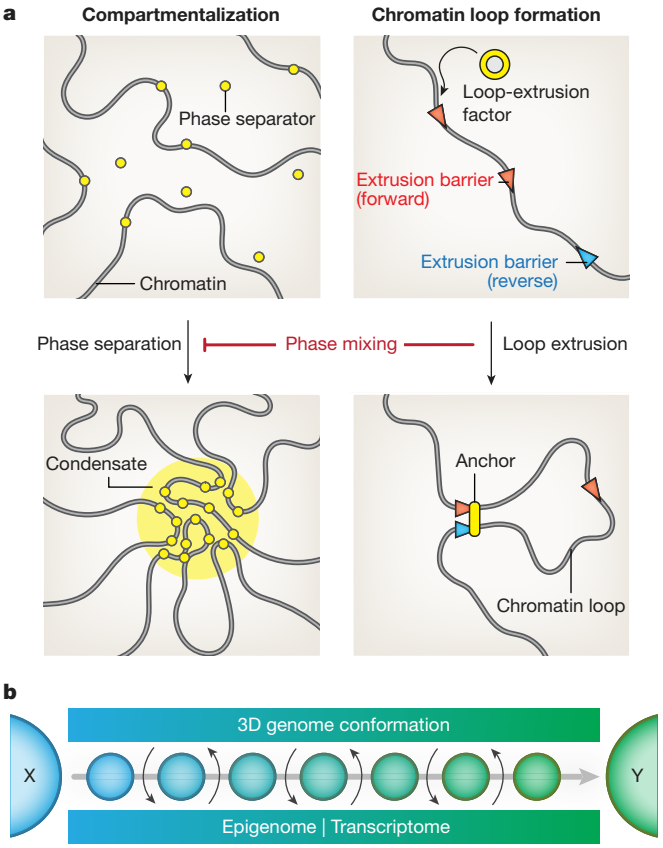

Fig. 3 | Compartmentalization and loop extrusion shape genome conformation. a, Left panels, spatial compartmentalization of the genome induced by a phase separator (for example, a transcription factor or other chromatin-associated protein). Condensates create 3D hubs that increase the local concentration of relevant associated factors and boost the efficiency of gene regulatory processes. Right panels, chromatin loop formation through extrusion. A loop-extrusion factor, such as cohesin (yellow ring), engages the chromatin to initiate extrusion of a chromatin loop (top right), similar to threading yarn through the eye of a needle, until it stops at an extrusion barrier (for example, chromatin-bound CTCF, depicted as coloured triangles). The binding orientation of CTCF (forward or reverse) is relevant for its interaction with cohesin, as loops are predominantly formed between two CTCF sites with convergent binding motifs. Stable loops (bottom right) are formed when the extrusion factor encounters a functional extrusion barrier on both sides (for example, a combination of a forward and reverse oriented CTCF site). See ref. [145] for an in-depth review. Note that loop extrusion can counteract compartmentalization to induce phase mixing. **b**, During cell-state transitions (exemplified by the conversion from state X to state Y), an iterative interplay between 3D genome conformation, the transcriptome and the epigenome ultimately establishes new cell identities.

when it is bound to DNA in a specific orientation[54,56,57]. Abrogating loop extrusion by depletion of cohesin (or its associated proteins) leads to a genome-wide loss of TAD organization and to increased intrachromosomal compartmentalization, indicating that loop extrusion and compartmentalization rely on different mechanisms that can act in an antagonistic fashion (Fig. 3a). Compartmentalization therefore appears to be the default mechanism of 3D genome folding, whereas loop extrusion establishes insulated genomic regions that are resistant to further compartmentalization[59]. Accordingly, in *Drosophila*, which lack a CTCF–cohesin-driven loop-extrusion mechanism, TADs are primarily formed by compartmentalization of active and inactive chromatin[60,61]. Finally, transcriptional activity and RNAPII occupancy have also been implicated in topological domain formation (reviewed in ref. [62]).

Although loop extrusion by CTCF–cohesin is critical for the formation of TADs in mammalian cells, a substantial number of domain boundaries remain unaffected by removal of CTCF[63,64]. Moreover, binding dynamics of CTCF fail to explain TAD border dynamics in time-resolved studies of transcription-factor-induced B-lymphocyte

reprogramming as well as across discrete stages of neural differentiation[34,65], which suggests that other modulators exist. As several transcription factors—including Krüppel-like factor 4 (KLF4) and octamer-binding transcription factor 4 (OCT4)—have been shown to interact with cohesin[66,67], it is tempting to speculate that they could also modulate genome conformation through loop extrusion. This notion is supported by the observation that the transcription factor Zelda is important for the establishment of specific TAD boundaries during *Drosophila* embryogenesis[68]. Rather than loop extrusion, multivalent transcription factor complexes with affinity for either active or inactive chromatin might form topological domains by creating protein bridges between different genomic loci[69]. Such a self-organizing mechanism would be akin to transcription factor–coactivator-induced condensate formation, which suggests that phase separation and compartmentalization could also have a role in TAD formation[61].

It is important to note that TADs do not follow a homogeneous definition. TADs vary widely in size and often present themselves as hierarchies of nested domains (which are sometimes referred to as sub-TADs[70]). Many TADs are formed by a chromatin loop and show characteristic punctate interaction signals at their apex in high-resolution Hi-C maps[54]. However, alongside these chromatin loop domains are TADs that are not formed by loops (that is, lacking punctate Hi-C signals), but that instead resemble A or B compartment domains. These TADs have recently been referred to as compartmental domains[61]. The two classes of TADs therefore appear to be formed by distinct mechanisms, but it remains unclear whether they also differ functionally.

Local interactions linking gene regulatory elements

Promoters and enhancers predominantly communicate within individual TADs[71–74], suggesting that TADs restrict the nuclear search space of regulatory elements. Indeed, the disruption or establishment of TAD boundaries may promote the formation of novel promoter–enhancer interactions, resulting in altered gene expression[34,65,75–77]. The view that TADs represent topological units of gene regulation is further supported by the observation that they are often covered by homogeneous chromatin signatures and exhibit concerted transcriptional responses[78,79]. Of note, the process of TAD formation itself, such as via cohesin-driven loop extrusion, can promote interactions between promoters and enhancers located in the vicinity of TAD boundaries[54].

Besides ubiquitous chromatin-associated proteins such as cohesin and Mediator[70,80], at least some lineage-restricted transcription factors—including LIM domain-binding protein 1 (LDB1), paired box 5 (PAX5), KLF4 and NANOG—can mediate enhancer–promoter interactions (for a review, see ref. [81]). Indeed, engineered protein oligomerization of LDB1 or YY1 has been shown to drive specific promoter–enhancer interactions, providing compelling evidence for an instructive role of transcription factors in this process[82,83]. Here again, phase separation induced by transcription factors or their coactivators provides a plausible mechanism for shaping fine-scale chromatin interactions within TADs. In this scenario, instead of the conventional stable lock-and-key model for specific promoter–enhancer interactions, transcription factors would induce dynamic contacts between regulatory sites through local condensate formation[11,36,39,84].

The process of transcription itself might also influence the proximity of promoters and enhancers in nuclear space. As well as a possible role for RNAPII-associated proteins in promoter–enhancer compartmentalization via phase separation (see above), dynamic RNAPII clusters have been proposed to form promoter–enhancer loops by recruiting specific gene loci[85]. Additionally, transcriptional activation has been shown to correlate with the nuclear mobility of regulatory elements, suggesting a positive feedback mechanism between transcription initiation and promoter–enhancer interactions[86].

Genome conformation and cell-fate decisions

The non-random spatial chromatin organization described above implies a role for 3D genome conformation in the transcription factor-driven control of gene regulation and, consequently, cell-fate decisions. In this section, we discuss our current understanding of the genome's form–function nexus, proposing that genome conformation can help to shape transcriptional plasticity by facilitating or impairing transcription factor function.

Specificity and heterogeneity of genome conformation

Genome conformation is partly cell-type-specific. For instance, the position of chromosome territories, including regions of intermingling, varies between cell types[87]. Moreover, gene association with the nuclear lamina changes during embryonic-stem-cell differentiation (13–27% of lamina-associated domains are dynamic[88]) and up to about 35% of the genome switches between A and B compartments during cellular differentiation or reprogramming[34,65,78,89,90]. Although TADs were first considered invariant across cell types, a growing body of evidence indicates that around 10–40% of TAD boundaries are cell-type-specific and that boundary insulation strength is plastic[34,54,65,91]. However, the most dynamic parameter of genome conformation is the promoter–enhancer interactome, in which the probability of specific promoter–enhancer interactions was found to exhibit a notable degree of cell-state specificity (that is, around 80% of promoters exhibit cell-type-specific contact frequencies)[65,92–94].

Genome conformation also demonstrated substantial cell-type specificity when analysed across the entire spectrum of genome-folding parameters in single cells[95]. Single-cell measurements are consistent with the view that compartments, TADs and loops exist as structural entities in individual cells, although with various degrees of cell-to-cell variability[96]. The A–B compartment status of genes in single cells appears to be fairly robust and exhibits a high degree of correlation with transcriptional activity in the corresponding bulk cell population[95,97], whereas TAD organization was found to be quite variable[95,97–99]. This suggests that within individual cells of a population, a given gene tends to remain associated with either the A or B compartment. By contrast, the same gene can fluctuate between TADs that differ in size and compaction, perhaps reflecting the continuous formation and dissolution of CTCF–cohesin loops observed in single-molecule imaging studies[100]. Notably, cohesin depletion does not abolish TAD boundary formation in single cells[99]; however, it appears essential for preferential boundary positioning[99], explaining the observed loss of distinct TAD boundaries in population-level Hi-C maps of cohesin-depleted cells[99,101].

Implications for transcriptional regulation

Recent studies integrating the dynamics of 3D genome folding and gene expression during cell differentiation or reprogramming further implicate genome conformation in transcriptional regulation[34,65,78,89–91,102]. For example, a time-course analysis of B lymphocytes undergoing synchronous transcription-factor-driven reprogramming into pluripotent stem cells reported notably close links between chromatin state, genome conformation and gene-expression dynamics[34]. Thus, as cells transit between states, overall changes in the spatial organization of their genomes are intimately coupled with local transcriptional and chromatin state dynamics—that is, whether genes in these regions become activated or repressed (Fig. 3b).

How then do the individual features of genome conformation influence gene transcription? Substantial evidence points towards physical proximity as the prime mechanism through which distal enhancers control promoter activity[5,11]. Accordingly, recent experiments using quantitative microscopy showed that sustained promoter–enhancer proximity is required for transgene activation in single *Drosophila* cells[103], and engineered promoter–enhancer interactions in mammalian cells are able to induce gene activation[82]. Interaction domains of insulated chromatin regions, such as TADs and loops, are thought to contribute to gene regulation by restricting the 3D search space in the nucleus of enhancers and their associated transcription factors to promote specific promoter–enhancer pairings[5,104]. Compelling evidence in favour of the importance of TADs for gene regulation comes from experiments in which a minimal promoter–reporter gene construct

was randomly integrated in the mouse genome, revealing similar tissue-specific expression patterns when integrations occurred within the same TAD[105]. Moreover, single-molecule transcription-factor imaging in live cells suggests that topological structures confine transcription factor-movement kinetics in the nucleus[106]. As predicted by these studies, experimental deletion of TAD boundaries or loop anchors (for example, through the deletion or inversion of CTCF sites) often results in altered expression of nearby genes[51,72,107–109]. In addition, chromosomal rearrangements can disrupt TAD boundaries, leading to inappropriate promoter–enhancer connections that can result in developmental defects or cancer[75,77] (reviewed in ref. [110]). For example, disruption of a TAD boundary at the *WNT6–IHH–EPHA4–PAX3* locus results in the ectopic activation of *WNT6*, *IHH* or *PAX3* by limb-specific enhancers of the *EPHA4* gene, as well as the concomitant loss of expression of *EPHA4* itself. This disruption of genome conformation and gene regulation causes digit malformations in humans[75]. TAD boundary disruption is also associated with repeat expansion disorders, such as fragile X syndrome[111].

As mentioned above, the removal of CTCF or cohesin from chromatin leads to a global disruption of TAD structure. Surprisingly, the marked loss of topological insulation is not accompanied by widespread transcriptional misregulation (fewer than 1,000 genes were affected, with predominantly small changes in expression), suggesting a more limited role of topological domain organization in restraining enhancer activity[63,112,113]. This indicates that TAD boundaries either predominantly fine-tune a cell's transcriptome or that they are important for the regulation of only a subset of genes. In support of the latter notion, acute cohesin depletion was most detrimental to the regulation of genes associated with large enhancer clusters (superenhancers), possibly owing to induction of strong superenhancer compartmentalization, which separates these regulatory elements from their target genes[101,114]. Additionally, the formation of new contacts between enhancers and promoters caused by CTCF–cohesin depletion might only result in altered gene regulation if the cell expresses the relevant transcription factors capable of recruiting the necessary transcriptional co-regulators. However, these studies were limited as they examined only short-term effects of CTCF–cohesin depletion on steady-state gene expression. In this regard, it is interesting that cohesin depletion was recently found to predominantly affect endotoxin-inducible gene expression in macrophages[115], indicating that TADs and loops are more important for the establishment of transcriptional networks than for their maintenance.

The functional relevance of the higher-order levels of genome conformation—such as A–B compartmentalization—for regulating gene expression is less clear, as manipulation of 3D genome folding at these levels remains difficult. However, clustering regions of similar biochemical activity around specific nuclear hallmarks or segregating them into chromosomal compartments has been proposed to boost the efficiency of gene regulatory processes by increasing the local concentration of relevant associated factors[116]. The observation that the position of a given gene in the nuclear space varies between individual cells (meaning that a gene has different long-range neighbours in every cell) has been used to argue that compartmentalization and radial gene positioning in the nucleus are too probabilistic to substantially affect transcriptional control[117]. However, the observed robust A–B compartmentalization of genes in single cells[95,97,116], possibly driven by transcription-factor-mediated condensate formation[39,40], suggests a more important role for chromosomal compartmentalization in transcriptional regulation than previously anticipated (see below).

Relating form to function

A central debate in the field revolves around the causal relationships between genome conformation, the chromatin landscape and transcription. The presence of specific topological structures is not sufficient to initiate gene regulatory processes. For instance, tethering a gene locus to the nuclear lamina does not necessarily induce gene repression[30] and promoter–enhancer interactions can occur without subsequent gene activation[118,119]. On the other hand, the process of transcription has detectable effects on genome conformation[68,86,120] and transcriptional activity can be used to accurately model features of 3D genome folding in silico[61]. However, experimental manipulation of 3D genome conformation has shown that inducing promoter–enhancer proximity or disrupting TAD boundary insulation can result in altered gene expression[75,82]. Conversely, inhibition of transcription does not disrupt existing genome conformation[121,122], nor does it prevent the establishment or maintenance of 3D genome organization during early embryonic development (see Box 1). In addition, a recent study showed that the PAX5 transcription factor is able to modify genome topology even in the absence of transcription[102]. Finally, changes in compartments and TAD borders often precede changes in gene expression during the conversion of somatic cells into pluripotent cells, explaining the different activation kinetics of the key pluripotency genes *Oct4*, *Nanog* and *Sox2*[34,123]. Therefore, it appears that during cell-fate conversions, function (transcription) frequently follows form (genome conformation).

What about the role of the chromatin landscape? Time-resolved analysis of B-cell reprogramming showed that transcription-factor-driven chromatin state changes (as visualized by H3K4me2 decoration) occurred either concomitantly with or before compartmentalization changes, whereas the reverse was rarely observed[34]. In addition, chromatin compaction or post-translational modifications of histones were sufficient to alter gene positioning or induce A–B compartment switching[30–32]. This implies that transcription-factor-mediated modifications of the epigenome can drive conformational changes—at least at the level of compartmentalization. Chromatin state dynamics, regulated by transcription factors and RNAPII, have also been suggested to have an active role in shaping TADs or intra-TAD interactions[61], a plausible mechanism considering the capacity of transcription factors and co-activators to promote local condensate formation[39,40,46,47].

In summary, whereas there is a continuous crosstalk between transcription, chromatin state and genome conformation (Fig. 3b), spatial genome (re)organization does not strictly require transcription. Additionally, alterations of genome conformation induced by transcription factors can create a permissive substrate for subsequent transcriptional changes. Together, these observations indicate that 3D genome folding holds instructive value for shaping gene-expression programs during cell-fate specification.

Transcription factor–genome conformation interplay

As discussed at the start of this review, cell identity is thought to emerge from a dynamic interplay between transcription factors, other chromatin-associated proteins and a spatially organized chromatin landscape (Fig. 1). Cell-fate-instructive transcription factors are ideally suited to confer spatiotemporal alterations of the 3D chromatin landscape given their cell-type-specific expression, responsiveness to signals, DNA sequence specificity and ability to pioneer non-permissive chromatin. Yet, genome conformation can restrain or promote the activity of transcription factors engaging with their targets to either impair or facilitate transcriptional plasticity. We envision four functionally distinct roles through which genome conformation and its interplay with transcription factors can help to control cell fate ('barrier', 'primer', 'optimizer' and 'facilitator' in Fig. 4a, b, c and d, respectively). Although they are useful for exemplifying how 3D genome organization can influence gene regulatory processes, the proposed mechanisms often function in an overlapping and concerted manner.

Genome conformation can act as a 'barrier' to phenotypic changes induced by signals that activate transcription factors, effectively stabilizing cell identity against perturbations. For example, the co-localization of genes targeted by co-operating transcription factors—recently visualized using technologies that can detect multi-way chromatin conformations[114,124,125]—has been proposed to safeguard the pluripotent stem cell state[41,48] (Fig. 4a) (also see Box 1). For instance, NANOG can directly induce spatial clustering of target genes to form 3D hubs[41,97,126]. Likewise, polycomb complexes form hubs that contain clusters of repressed genes[43,48]. The spatial proximity

Box 1

Genome conformation during embryogenesis and in pluripotent cells

In 2017, several groups published Hi-C analyses of genome conformation in mammalian germ cells and during the earliest stages of development[98,146–149]. Whereas all cardinal principles of 3D chromatin folding (that is, A and B compartments, TADs and loops) were readily detectable in sperm[149], these features were much weaker (although detectable) in individual oocytes[148]. At the zygote stage, the paternal nucleus maintains both TADs and A and B compartments, whereas the maternal nucleus specifically loses the latter[98,147], indicating that separate processes drive TAD formation and A–B compartmentalization. During subsequent stages of early mouse embryogenesis (two-to-eight-cell stage) topological features can be detected in both parental genomes, and become progressively stronger[98,146–148]. Transcription of the genome is first initiated at the two-cell stage (referred to as zygotic genome activation (ZGA)). Transcription inhibitors block ZGA but do not disrupt the build-up in genome conformation[146,147]. Similar analyses performed in developing *Drosophila*[68] or zebrafish[150] embryos confirmed that establishing 3D chromatin folding does not require ZGA.

The first pluripotent stem cells originate in the inner cell mass of the blastocyst and are the source of embryonic stem cells. Analysing genome conformation in these highly plastic cells and in induced pluripotent stem cells has revealed unique aspects of 3D genome organization in pluripotent cells when compared to somatic cells. For example, large CTCF-mediated structural loops are generally less abundant in pluripotent cells[91], whereas regions residing in the pluripotent B compartment engage in fewer specific contacts[41], show weaker overall compartmentalization[34,41,65] and interact less robustly with the nuclear lamina[88]. These observations indicate that repression of genes via chromatin compartmentalization is less efficient in pluripotent stem cells, possibly owing to a weaker HP1α-driven phase separation[49]. The weaker B compartmentalization in pluripotent cells might explain the finding that they contain a larger number of TADs than differentiated cells[34,65], perhaps because weaker B compartmentalization allows for enhanced local TAD formation via loop extrusion (Fig. 3a). In addition, differentiation-associated genes in pluripotent stem cells, such as Hox genes, engage in long-range 3D interaction networks mediated by polycomb proteins to ensure a silent but poised state[48]. Together, these results indicate that specialized genome conformations can contribute to the plasticity of pluripotent cells, aside from mechanisms that operate at the nucleosomal level[151].

of genes controlled by the same regulatory factors is therefore thought to increase the robustness by which their active or inactive states are maintained[127]. Mechanistically, regulatory proteins could form such 3D hubs via homotypic dimerization or through interactions between low-complexity domains[39,40]. This in turn would evoke (transient) phase separation and drive compartmentalization of regulated genes. Alternatively, genome conformation could directly impede induced cell-fate changes, as exemplified by topological domains that restrain the activity of transcription factors bound at their target regulatory elements[110]. In addition, as the euchromatin–heterochromatin (or A–B compartment) phase-separation border appears to act as a barrier to protein diffusion[50], chromatin compartmentalization might prevent inclusion of activating transcription factor complexes into droplet-like B compartment domains[38]. This scenario provides an explanation for the almost instant activation of the pluripotency gene *Oct4*—unlike *Sox2*—by the Yamanaka transcription factors during B-lymphocyte reprogramming, as *Oct4* already resides in an A compartment condensate accessible to the transcription factors, whereas *Sox2* is incorporated in the B compartment[34] (Fig. 4a).

Chromatin conformation can also act as a 'primer' to provide transcription factors with opportunities to destabilize cellular states (Fig. 4b). Pre-formed promoter–enhancer interactions can create a permissive regulatory landscape that primes inactive genes for rapid activation upon receiving a specific stimulus[5]. Similar spatial priming might also occur through the positioning of an inactive gene in the A compartment (for example, *Oct4* in Fig. 4a). In addition, regulatory complexes could use genome conformation to more-efficiently maintain genes in a developmentally primed state[128,129]. For example, polycomb-mediated 3D hubs in pluripotent cells enhance repression of differentiation-associated genes (a barrier function, see above) but at the same time ensure that they are maintained in a poised state that allows for a rapid de-repression when encountering specific differentiation signals (a bivalent state)[48,130,131] (Fig. 4b). More generally, 3D genome conformation in pluripotent stem cells exhibits unique features that could explain their extraordinary developmental plasticity (Box 1). Along these lines, topological reorganization mediated by the abundant chromatin-associated high mobility group B2 (HMGB2) protein was suggested to prime cells for activation of a senescence program[132].

Genome topology can also act as a local 'optimizer' of gene expression during transcription-factor-induced cell state transitions (Fig. 4c). For example, during cell reprogramming, contact frequencies between promoters and enhancers of pluripotency genes must be accurately established to ensure correct transcriptional outputs[133]. During B lymphocyte development, the binding of specific transcription factors to immunoglobulin gene enhancers ensures that individual variable (V), diversity (D) or joining (J) segments have equal opportunities to recombine, thus maximizing antigen receptor diversity[134]. This is achieved through locus contraction, which enables V segments to move into close proximity to the recombination centre—even when located more than a million bases away[135,136] (Fig. 4c). Impaired locus contraction does not prevent recombination and lymphocyte maturation; instead, it leads to highly skewed recombination of proximal V segments in the linear genome[137–139]. Notably, before recombination of the immunoglobulin light chain locus, transcription factors bind enhancers inside the recombination centre and initiate locus contraction through mostly random interactions with the megabase-scale V-gene region. In a process called enhancer focusing, differentiation signals then activate additional transcription factors to iteratively optimize these interactions and establish a highly coordinated enhancer interactome that calibrates V-segment recombination frequencies[134,140] (Fig. 4c).

Finally, topological reorganization itself could act as a 'facilitator', evoking transcriptional plasticity without relying on the activation of specific signal-responsive regulatory factors. For example, tethering the self-associating domain of LDB1 to the β-globin promoter is sufficient to induce interactions with LDB1 complexes bound at the β-globin enhancer, leading to gene activation[82] (Fig. 4d). This provides a proof-of-principle for the concept of chromatin looping facilitating cell-fate conversions. As genome conformation is dynamic and to a certain extent probabilistic, the stochastic occurrence of certain promoter–enhancer interactions might allow existing transcription factor complexes to alter gene expression in only a subset of cells[141]. Spontaneous variations of genome conformation may thus induce cell-fate changes by creating new opportunities for established transcription factor networks to shape gene-expression programs, including disease-associated transcriptional changes[110]. Such a mechanism has been described for haematopoietic cells containing a rearrangement in chromosome 3 that incorporates a *GATA2* enhancer into the TAD containing the *EVI1* oncogene (Fig. 4d). This newly created genome configuration induces *EVI1* overexpression and a decrease of *GATA2* levels, leading to cell transformation and cancer[142].

In summary, changes in cell-state-specific 3D genome conformation can create either barriers or opportunities for regulatory factors to safeguard or destabilize a cell's transcriptome. Iterative interactions of

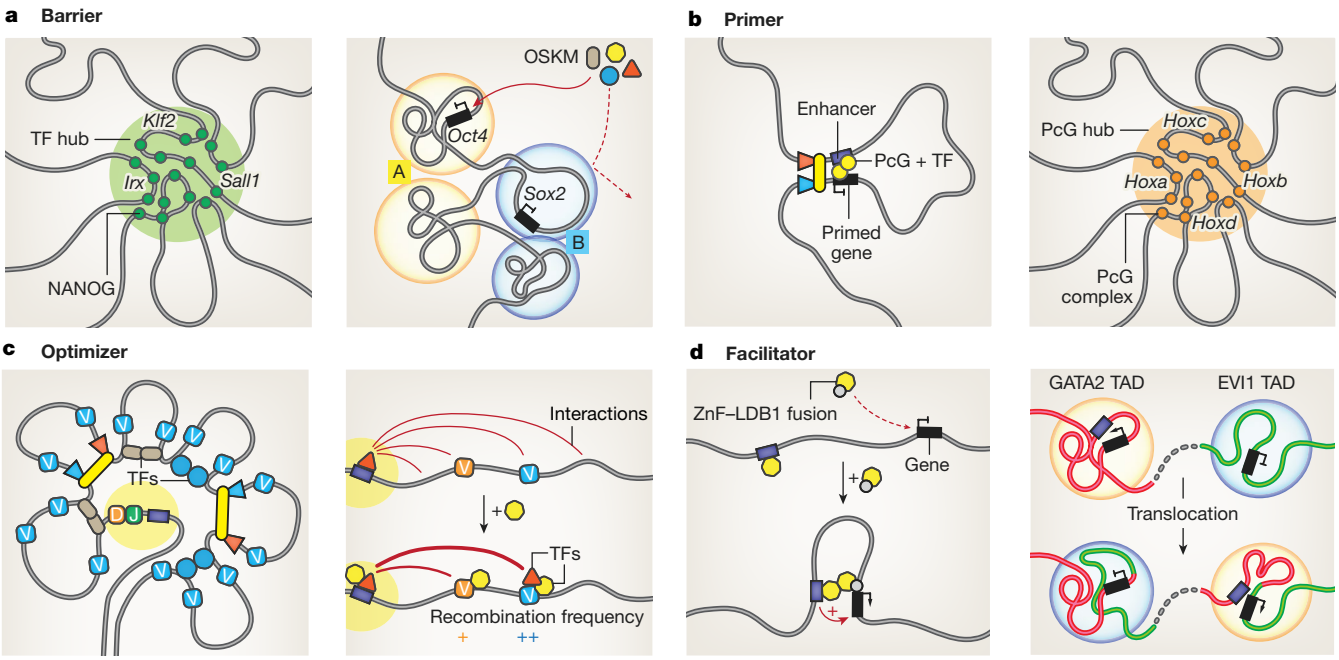

Fig. 4 | Scenarios and examples of how genome conformation helps to shape cell identity. Four scenarios with examples of how 3D genome conformation may modulate transcription-factor-induced cell-state transitions (that is, a signalling-dependent change from state X to state Y). **a**, Genome conformation functions as a barrier for phenotypic change. Left, the NANOG transcription factor forms a hub (for example, through condensate formation (green shading)) to cluster its target genes (*Klf2*, *Sall1 and Irx* are shown) and to robustly maintain their expression levels[41]. Right, during OSKM-induced B lymphocyte reprogramming, the OSKM transcription factors are able to access the *Oct4* locus, which locates to the phase-separated A compartment. However, OSKM is excluded from the *Sox2* locus located in the B compartment. This results in faster activation of *Oct4* than *Sox2*[34]. **b**, Genome conformation functions as a primer. Left, combinations of transcription factors can facilitate chromatin loop formation (here formed by loop extrusion, as indicated by the yellow ring and convergent triangles, see Fig. 3a) before actual gene activation. Right, polycomb group (PcG) proteins form hubs to maintain their genes in a silent-but-poised state (various Hox gene clusters[48] are shown). **c**, Genome conformation functions as an optimizer. Left, during B lymphocyte differentiation, transcription factors induce locus contraction (shown as a 'rosette', in part mediated by loop extrusion) at immunoglobulin loci to induce spatial proximity between the various variable (V) genes and the enhancers (depicted as a purple rectangle next to the D and J gene segments) in the recombination centre (yellow shaded region), optimizing individual V-gene accessibility for recombination[136]. Right, interactions between V-gene enhancers and promoters at immunoglobulin loci in B-cell progenitors are initially imprecise and random. Signal-responsive transcription factors (indicated by the yellow heptagon) then focus the enhancers to be more specific for certain V genes, reflected by individual V-gene recombination efficiencies[140] (as indicated by +). **d**, Genome conformation functions as a facilitator. Left, tethering of a ZnF–LDB1 fusion protein to the β-globin promoter induces promoter–enhancer loop formation and, subsequently, gene activation using the existing transcription factor network[82]. Right, chromosomal rearrangements incorporate a *GATA2* enhancer (purple triangle) into the *EVI1*-containing TAD, resulting in *EVI1* oncogene activation, *GATA2* silencing and malignant transformation[142]. ZnF, zinc finger.

transcription factors with cofactors and the epigenome in the context of specific genome conformations then catalyse self-organizing processes (for example, the formation of phase-separated condensates) to specify new gene-expression programs and cell identities. Such gradual but progressive changes in genome topology and gene expression are consistent with recent evidence obtained from single-cell gene-expression analyses of mouse and human haematopoietic cells. This body of work suggests that multipotent progenitors separate into different lineages through a continuum of intermediate cell states, rather than via abrupt binary switches[143].

Perspectives

The study of the 3D genome is rapidly becoming an integral aspect in our understanding of cell-fate specification as it occurs during embryonic development, adult cell differentiation and cell reprogramming. Recent discoveries have shed light on the mechanisms that drive our genome to adopt specific conformations, but much remains to be investigated. Further work is needed to dissect the molecular details that underlie loop extrusion and compartmentalization, two seemingly opposing processes that represent the engines of 3D chromatin organization in mammals. This is needed to understand how transcription factors and other regulatory proteins interact with these topological engines to shape the gene-expression programs that determine a cell's identity. For example, do cell-fate-instructive transcription factors have unique abilities to alter or exploit genome conformation[34,102], and if so, is this related to their capacity to act as pioneers, binding to nucleosomal DNA[6]? Perhaps it is the ability of cell-fate-instructive transcription factors to modulate 3D genome organization at multiple levels, including the formation of phase-separated condensates[39], that endows them with their unique cell reprogramming capacity.

The concept of phase separation is attracting attention, as it represents a simple and intuitive mechanism that can explain the tendency of the genome to compartmentalize similar biochemical processes. Nevertheless, additional experiments are required to test whether phase-separated condensates are essential for establishing and maintaining genome conformation, from large-scale A–B compartmentalization down to local promoter–enhancer interactions. Additionally, it is unclear whether there are functionally distinct condensates formed by different transcription factors and co-activators, and—if so—what drives their specificity, and how many types of chromatin-containing condensates exist. Selective interactions between low-complexity disordered transcription-factor domains provide a possible mechanism for creating spatial hubs between specific genes and their regulatory elements[39,40]. Future work aimed at elucidating the biochemical and structural basis of transcription-factor-driven phase separation may reveal new layers of regulatory information encoded in the structure of these factors.

Although there is widespread agreement that genome form and function are intimately connected, their causal relationship remains controversial. Tackling the form–function nexus will require sophisticated

new approaches, such as programmable editing of 3D genome organization[144] and technologies capable of simultaneously measuring changes in genome conformation and gene transcription in single cells. Another area of interest is the development of methods to systematically investigate the causal relationship between condensate formation and parameters of genome conformation. Importantly, future research addressing how cells decide their fate must include the temporal dimension. Thus, studying the cellular interpretation of genomic information at the 3D level alone is no longer sufficient: it should be considered as a '4D affair' in both space and time.

Received: 15 October 2018; Accepted: 19 March 2019;
Published online 15 May 2019.

1. Davidson, E. H. Emerging properties of animal gene regulatory networks. *Nature* **468**, 911–920 (2010).
2. Vaquerizas, J. M., Kummerfeld, S. K., Teichmann, S. A. & Luscombe, N. M. A census of human transcription factors: function, expression and evolution. *Nat. Rev. Genet.* **10**, 252–263 (2009).
3. Lambert, S. A. et al. The human transcription factors. *Cell* **172**, 650–665 (2018).
4. Kundaje, A. et al. Integrative analysis of 111 reference human epigenomes. *Nature* **518**, 317–330 (2015).
5. de Laat, W. & Duboule, D. Topology of mammalian developmental enhancers and their regulatory landscapes. *Nature* **502**, 499–506 (2013).
6. Zaret, K. S. & Mango, S. E. Pioneer transcription factors, chromatin dynamics, and cell fate control. *Curr. Opin. Genet. Dev.* **37**, 76–81 (2016).
7. Spitz, F. & Furlong, E. E. Transcription factors: from enhancer binding to developmental control. *Nat. Rev. Genet.* **13**, 613–626 (2012).
8. Voss, T. C. & Hager, G. L. Dynamic regulation of transcriptional states by chromatin and transcription factors. *Nat. Rev. Genet.* **15**, 69–81 (2014).
9. Dekker, J. & Mirny, L. The 3D genome as moderator of chromosomal communication. *Cell* **164**, 1110–1121 (2016).
10. Bonev, B. & Cavalli, G. Organization and function of the 3D genome. *Nat. Rev. Genet.* **17**, 661–678 (2016).
11. Furlong, E. E. M. & Levine, M. Developmental enhancers and chromosome topology. *Science* **361**, 1341–1345 (2018).
12. Novikoff, A. B. The concept of integrative levels and biology. *Science* **101**, 209–215 (1945).
13. Graf, T. & Enver, T. Forcing cells to change lineages. *Nature* **462**, 587–594 (2009).
14. Denker, A. & de Laat, W. The second decade of 3C technologies: detailed insights into nuclear organization. *Genes Dev.* **30**, 1357–1382 (2016).
15. Yadav, T., Quivy, J. P. & Almouzni, G. Chromatin plasticity: a versatile landscape that underlies cell fate and identity. *Science* **361**, 1332–1336 (2018).
16. Cremer, T. & Cremer, C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat. Rev. Genet.* **2**, 292–301 (2001).
17. Bronshtein, I. et al. Exploring chromatin organization mechanisms through its dynamic properties. *Nucleus* **7**, 27–33 (2016).
18. Branco, M. R. & Pombo, A. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLoS Biol.* **4**, e138 (2006).
19. Quinodoz, S. A. et al. Higher-order inter-chromosomal hubs shape 3D genome organization in the nucleus *Cell* **174**, 744–757 (2018).
20. Spilianakis, C. G., Lalioti, M. D., Town, T., Lee, G. R. & Flavell, R. A. Interchromosomal associations between alternatively expressed loci. *Nature* **435**, 637–645 (2005).
21. Lomvardas, S. et al. Interchromosomal interactions and olfactory receptor choice. *Cell* **126**, 403–413 (2006).
22. Apostolou, E. & Thanos, D. Virus infection induces NF-κB-dependent interchromosomal associations mediating monoallelic *IFNβ* gene expression. *Cell* **134**, 85–96 (2008).
23. Monahan, K., Horta, A. & Lomvardas, S. LHX2- and LDB1-mediated trans interactions regulate olfactory receptor choice. *Nature* **565**, 448–453 (2019).
24. Schoenfelder, S. et al. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. *Nat. Genet.* **42**, 53–61 (2010).
25. Yusufzai, T. M., Tagami, H., Nakatani, Y. & Felsenfeld, G. CTCF tethers an insulator to subnuclear sites, suggesting shared insulator mechanisms across species. *Mol. Cell* **13**, 291–298 (2004).
26. Brown, K. E. et al. Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. *Cell* **91**, 845–854 (1997).
27. Lieberman-Aiden, E. et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289–293 (2009).
A landmark paper reporting the development of the widely used Hi-C technology and describing the division of the human genome into two topological compartments, A and B.
28. Wijchers, P. J. et al. Characterization and dynamics of pericentromere-associated domains in mice. *Genome Res.* **25**, 958–969 (2015).
29. Vieux-Rochas, M., Fabre, P. J., Leleu, M., Duboule, D. & Noordermeer, D. Clustering of mammalian *Hox* genes with other H3K27me3 targets within an active nuclear domain. *Proc. Natl Acad. Sci. USA* **112**, 4672–4677 (2015).
30. van Steensel, B. & Belmont, A. S. Lamina-associated domains: links with chromosome architecture, heterochromatin, and gene repression. *Cell* **169**, 780–791 (2017).
31. Therizols, P. et al. Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells. *Science* **346**, 1238–1242 (2014).
32. Wijchers, P. J. et al. Cause and consequence of tethering a subTAD to different nuclear compartments. *Mol. Cell* **61**, 461–473 (2016).
33. Harr, J. C. et al. Directed targeting of chromatin to the nuclear lamina is mediated by chromatin state and A-type lamins. *J. Cell Biol.* **208**, 33–52 (2015).
34. Stadhouders, R. et al. Transcription factors orchestrate dynamic interplay between genome topology and gene regulation during cell reprogramming. *Nat. Genet.* **50**, 238–249 (2018).
The first study to describe the dynamics of mammalian genome conformation as somatic cells reprogram into pluripotent cells, during which a substantial number of loci, including those of key pluripotency factors, undergo topological changes in advance of transcriptional changes.
35. Maeshima, K., Ide, S., Hibino, K. & Sasai, M. Liquid-like behavior of chromatin. *Curr. Opin. Genet. Dev.* **37**, 36–45 (2016).
36. Hnisz, D., Shrinivas, K., Young, R. A., Chakraborty, A. K. & Sharp, P. A. A phase separation model for transcriptional control. *Cell* **169**, 13–23 (2017).
37. Hyman, A. A., Weber, C. A. & Jülicher, F. Liquid–liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* **30**, 39–58 (2014).
38. Shin, Y. et al. Liquid nuclear condensates mechanically sense and restructure the genome *Cell* **175**, 1481–1491 (2018).
39. Boija, A. et al. Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell* **175**, 1842–1855 (2018).
40. Chong, S. et al. Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science* **361**, eaar2555 (2018).
Together with ref. [39], this paper implicates transcription factors as direct mediators of phase separation.
41. de Wit, E. et al. The pluripotent genome in three dimensions is shaped around pluripotency factors. *Nature* **501**, 227–231 (2013).
42. Liu, Z. et al. 3D imaging of Sox2 enhancer clusters in embryonic stem cells. *eLife* **3**, e04236 (2014).
43. Bantignies, F. et al. Polycomb-dependent regulatory contacts between distant Hox loci in *Drosophila*. *Cell* **144**, 214–226 (2011).
44. Boehning, M. et al. RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nat. Struct. Mol. Biol.* **25**, 833–840 (2018).
45. Lu, H. et al. Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. *Nature* **558**, 318–323 (2018).
46. Cho, W. K. et al. Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. *Science* **361**, 412–415 (2018).
47. Sabari, B. R. et al. Coactivator condensation at super-enhancers links phase separation and gene control. *Science* **361**, eaar3958 (2018).
48. Schoenfelder, S. et al. Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. *Nat. Genet.* **47**, 1179–1186 (2015).
49. Larson, A. G. et al. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. *Nature* **547**, 236–240 (2017).
50. Strom, A. R. et al. Phase separation drives heterochromatin domain formation. *Nature* **547**, 241–245 (2017).
51. Nora, E. P. et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* **485**, 381–385 (2012).
52. Dixon, J. R. et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376–380 (2012).
53. Sexton, T. et al. Three-dimensional folding and functional organization principles of the *Drosophila* genome. *Cell* **148**, 458–472 (2012).
Together with refs.[51,52], this study reports the existence of topologically associating domains as a principle of 3D genome organization.
54. Rao, S. S. et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665–1680 (2014).
An improved Hi-C protocol for high-resolution analysis that allows identification of chromatin loops and further refines A–B compartment segmentation.
55. Vietri Rudan, M. et al. Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture. *Cell Reports* **10**, 1297–1309 (2015).
56. Fudenberg, G. et al. Formation of chromosomal domains by loop extrusion. *Cell Reports* **15**, 2038–2049 (2016).
57. Sanborn, A. L. et al. Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *Proc. Natl Acad. Sci. USA* **112**, E6456–E6465 (2015).
58. Alipour, E. & Marko, J. F. Self-organization of domain structures by DNA-loop-extruding enzymes. *Nucleic Acids Res.* **40**, 11202–11212 (2012).
59. Nuebler, J., Fudenberg, G., Imakaev, M., Abdennur, N. & Mirny, L. A. Chromatin organization by an interplay of loop extrusion and compartmental segregation. *Proc. Natl Acad. Sci. USA* **115**, E6697–E6706 (2018).
60. Ulianov, S. V. et al. Active chromatin and transcription play a key role in chromosome partitioning into topologically associating domains. *Genome Res.* **26**, 70–84 (2016).
61. Rowley, M. J. et al. Evolutionarily conserved principles predict 3D chromatin organization *Mol. Cell* **67**, 837–852 (2017).
62. Rada-Iglesias, A., Grosveld, F. G. & Papantonis, A. Forces driving the three-dimensional folding of eukaryotic genomes. *Mol. Syst. Biol.* **14**, e8214 (2018).
63. Nora, E. P. et al. Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization *Cell* **169**, 930–944 (2017).

Degron-mediated CTCF depletion in embryonic stem cells is used to show that the factor is essential for creating and maintaining TAD borders but not A–B compartments.
64. Wutz, G. et al. Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. *EMBO J.* **36**, 3573–3599 (2017).
65. Bonev, B. et al. Multiscale 3D genome rewiring during mouse neural development. *Cell* **171**, 557–572 (2017).
66. Wei, Z. et al. Klf4 organizes long-range chromosomal interactions with the *Oct4* locus in reprogramming and pluripotency. *Cell Stem Cell* **13**, 36–47 (2013).
67. Abboud, N. et al. A cohesin–OCT4 complex mediates *Sox* enhancers to prime an early embryonic lineage. *Nat. Commun.* **6**, 6749 (2015).
68. Hug, C. B., Grimaldi, A. G., Kruse, K. & Vaquerizas, J. M. Chromatin architecture emerges during zygotic genome activation independent of transcription. *Cell* **169**, 216–228 (2017).
69. Brackley, C. A., Johnson, J., Kelly, S., Cook, P. R. & Marenduzzo, D. Simulated binding of transcription factors to active and inactive regions folds human chromosomes into loops, rosettes and topological domains. *Nucleic Acids Res.* **44**, 3503–3512 (2016).
70. Phillips-Cremins, J. E. et al. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. *Cell* **153**, 1281–1295 (2013).
71. Shen, Y. et al. A map of the *cis*-regulatory sequences in the mouse genome. *Nature* **488**, 116–120 (2012).
72. Dowen, J. M. et al. Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. *Cell* **159**, 374–387 (2014).
73. Schoenfelder, S. et al. The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. *Genome Res.* **25**, 582–597 (2015).
74. Sun, F. et al. Promoter–enhancer communication occurs primarily within insulated neighborhoods. *Mol. Cell* **73**, 250–263 (2019).
75. Lupiáñez, D. G. et al. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. *Cell* **161**, 1012–1025 (2015).
The first study that convincingly links disruption of TAD structure to transcriptional deregulation as well as mouse and human limb malformations.
76. Flavahan, W. A. et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature* **529**, 110–114 (2016).
77. Hnisz, D. et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. *Science* **351**, 1454–1458 (2016).
78. Dixon, J. R. et al. Chromatin architecture reorganization during stem cell differentiation. *Nature* **518**, 331–336 (2015).
79. Le Dily, F. et al. Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. *Genes Dev.* **28**, 2151–2162 (2014).
80. Kagey, M. H. et al. Mediator and cohesin connect gene expression and chromatin architecture. *Nature* **467**, 430–435 (2010).
81. Andrey, G. & Mundlos, S. The three-dimensional genome: regulating gene expression during pluripotency and development. *Development* **144**, 3646–3658 (2017).
82. Deng, W. et al. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. *Cell* **149**, 1233–1244 (2012).
The first conclusive evidence that forcing enhancer–promoter interactions is sufficient to alter gene expression.
83. Weintraub, A. S. et al. YY1 is a structural regulator of enhancer–promoter loops. *Cell* **171**, 1573–1588 (2017).
84. Liu, Z. & Tjian, R. Visualizing transcription factor dynamics in living cells. *J. Cell Biol.* **217**, 1181–1191 (2018).
85. Papantonis, A. & Cook, P. R. Transcription factories: genome organization and gene regulation. *Chem. Rev.* **113**, 8683–8705 (2013).
86. Gu, B. et al. Transcription-coupled changes in nuclear mobility of mammalian *cis*-regulatory elements. *Science* **359**, 1050–1055 (2018).
87. Fraser, J., Williamson, I., Bickmore, W. A. & Dostie, J. An overview of genome organization and how we got there: from FISH to Hi-C. *Microbiol. Mol. Biol. Rev.* **79**, 347–372 (2015).
88. Peric-Hupkes, D. et al. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol. Cell* **38**, 603–613 (2010).
89. Hu, G. et al. Transformation of accessible chromatin and 3D nucleome underlies lineage commitment of early T cells. *Immunity* **48**, 227–242 (2018).
90. Krijger, P. H. et al. Cell-of-origin-specific 3D genome structure acquired during somatic cell reprogramming. *Cell Stem Cell* **18**, 597–610 (2016).
91. Pekowska, A. et al. Gain of CTCF-anchored chromatin loops marks the exit from naive pluripotency. *Cell Syst.* **7**, 482–495 (2018).
92. Kieffer-Kwon, K. R. et al. Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. *Cell* **155**, 1507–1520 (2013).
93. Javierre, B. M. et al. Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. *Cell* **167**, 1369–1384 (2016).
94. Zhang, Y. et al. Chromatin connectivity maps reveal dynamic promoter–enhancer long-range associations. *Nature* **504**, 306–310 (2013).
95. Tan, L., Xing, D., Chang, C. H., Li, H. & Xie, X. S. Three-dimensional genome structures of single diploid human cells. *Science* **361**, 924–928 (2018).
96. Ulianov, S. V., Tachibana-Konwalski, K. & Razin, S. V. Single-cell Hi-C bridges microscopy and genome-wide sequencing approaches to study 3D chromatin organization. *BioEssays* **39**, 1700104 (2017).
97. Stevens, T. J. et al. 3D structures of individual mammalian genomes studied by single-cell Hi-C. *Nature* **544**, 59–64 (2017).
98. Flyamer, I. M. et al. Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. *Nature* **544**, 110–114 (2017).
99. Bintu, B. et al. Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science* **362**, eaau1783 (2018).
Super-resolution imaging is used to show that TADs exist in single cells with variable boundaries, and cohesin is shown to specifically control preferential boundary positioning.
100. Hansen, A. S., Cattoglio, C., Darzacq, X. & Tjian, R. Recent evidence that TADs and chromatin loops are dynamic structures. *Nucleus* **9**, 20–32 (2018).
101. Rao, S. S. P. et al. Cohesin loss eliminates all loop domains. *Cell* **171**, 305–320 (2017).
102. Johanson, T. M. et al. Transcription-factor-mediated supervision of global genome architecture maintains B cell identity. *Nat. Immunol.* **19**, 1257–1264 (2018).
103. Chen, H. et al. Dynamic interplay between enhancer-promoter topology and gene activity. *Nat. Genet.* **50**, 1296–1303 (2018).
104. Andrey, G. et al. A switch between topological domains underlies *HoxD* genes collinearity in mouse limbs. *Science* **340**, 1234167 (2013).
105. Symmons, O. et al. Functional and topological characteristics of mammalian regulatory domains. *Genome Res.* **24**, 390–400 (2014).
106. Liu, H. et al. Visualizing long-term single-molecule dynamics in vivo by stochastic protein labeling. *Proc. Natl Acad. Sci. USA* **115**, 343–348 (2018).
107. de Wit, E. et al. CTCF binding polarity determines chromatin looping. *Mol. Cell* **60**, 676–684 (2015).
108. Guo, Y. et al. CRISPR inversion of CTCF sites alters genome topology and enhancer/promoter function. *Cell* **162**, 900–910 (2015).
109. Narendra, V. et al. CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation. *Science* **347**, 1017–1021 (2015).
110. Krijger, P. H. & de Laat, W. Regulation of disease-associated gene expression in the 3D genome. *Nat. Rev. Mol. Cell Biol.* **17**, 771–782 (2016).
111. Sun, J. H. et al. Disease-associated short tandem repeats co-localize with chromatin domain boundaries. *Cell* **175**, 224–238 (2018).
112. Schwarzer, W. et al. Two independent modes of chromatin organization revealed by cohesin removal. *Nature* **551**, 51–56 (2017).
In vivo depletion of the cohesin loading factor Nipbl leads to genome-wide loss of TAD organization, whereas A–B compartmentalization is strengthened, suggesting that TAD formation and compartmentalization are separate antagonistic processes.
113. Haarhuis, J. H. I. et al. The cohesin release factor WAPL restricts chromatin loop extension. *Cell* **169**, 693–707 (2017).
Dissection of the functional role of cohesin loading and release factors in chromatin loop formation and their size.
114. Beagrie, R. A. et al. Complex multi-enhancer contacts captured by genome architecture mapping. *Nature* **543**, 519–524 (2017).
115. Cuartero, S. et al. Control of inducible gene expression links cohesin to hematopoietic progenitor self-renewal and differentiation. *Nat. Immunol.* **19**, 932–941 (2018).
Demonstration that the induction of inflammatory response genes in haematopoietic cells is highly dependent on cohesin.
116. Wang, S. et al. Spatial organization of chromatin domains and compartments in single chromosomes. *Science* **353**, 598–602 (2016).
117. Krijger, P. H. & de Laat, W. Identical cells with different 3D genomes; cause and consequences? *Curr. Opin. Genet. Dev.* **23**, 191–196 (2013).
118. Ghavi-Helm, Y. et al. Enhancer loops appear stable during development and are associated with paused polymerase. *Nature* **512**, 96–100 (2014).
119. Jin, F. et al. A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* **503**, 290–294 (2013).
120. Li, L. et al. Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. *Mol. Cell* **58**, 216–231 (2015).
121. Palstra, R. J. et al. Maintenance of long-range DNA interactions after inhibition of ongoing RNA polymerase II transcription. *PLoS ONE* **3**, e1661 (2008).
122. Mitchell, J. A. & Fraser, P. Transcription factories are nuclear subcompartments that remain in the absence of transcription. *Genes Dev.* **22**, 20–25 (2008).
123. Apostolou, E. et al. Genome-wide chromatin interactions of the *Nanog* locus in pluripotency, differentiation, and reprogramming. *Cell Stem Cell* **12**, 699–712 (2013).
124. Oudelaar, A. M. et al. Single-allele chromatin interactions identify regulatory hubs in dynamic compartmentalized domains. *Nat. Genet.* **50**, 1744–1751 (2018).
125. Allahyar, A. et al. Enhancer hubs and loop collisions identified from single-allele topologies. *Nat. Genet.* **50**, 1151–1160 (2018).
126. Denholtz, M. et al. Long-range chromatin contacts in embryonic stem cells reveal a role for pluripotency factors and polycomb proteins in genome organization. *Cell Stem Cell* **13**, 602–616 (2013).
127. Corrales, M. et al. Clustering of *Drosophila* housekeeping promoters facilitates their expression. *Genome Res.* **27**, 1153–1161 (2017).
128. Bernstein, B. E. et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326 (2006).
129. Stadhouders, R. et al. Control of developmentally primed erythroid genes by combinatorial co-repressor actions. *Nat. Commun.* **6**, 8893 (2015).
130. Cruz-Molina, S. et al. PRC2 facilitates the regulatory topology required for poised enhancer function during pluripotent stem cell differentiation. *Cell Stem Cell* **20**, 689–705 (2017).

131. Mas, G. et al. Promoter bivalency favors an open chromatin architecture in embryonic stem cells. *Nat. Genet.* **50**, 1452–1462 (2018).
132. Zirkel, A. et al. HMGB2 loss upon senescence entry disrupts genomic organization and induces CTCF clustering across cell types. *Mol. Cell* **70**, 730–744 (2018).
133. Beagan, J. A. et al. Local genome topology can exhibit an incompletely rewired 3D-folding state during somatic cell reprogramming. *Cell Stem Cell* **18**, 611–624 (2016).
134. Ribeiro de Almeida, C., Hendriks, R. W. & Stadhouders, R. Dynamic control of long-range genomic interactions at the immunoglobulin κ light-chain locus. *Adv. Immunol.* **128**, 183–271 (2015).
135. Kosak, S. T. et al. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* **296**, 158–162 (2002).
136. Jhunjhunwala, S. et al. The 3D structure of the immunoglobulin heavy-chain locus: implications for long-range genomic interactions. *Cell* **133**, 265–279 (2008).
137. Fuxa, M. et al. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes Dev.* **18**, 411–422 (2004).
138. Guo, C. et al. CTCF-binding elements mediate control of V(D)J recombination. *Nature* **477**, 424–430 (2011).
139. Ribeiro de Almeida, C. et al. The DNA-binding protein CTCF limits proximal Vκ recombination and restricts κ enhancer interactions to the immunoglobulin κ light chain locus. *Immunity* **35**, 501–513 (2011).
140. Stadhouders, R. et al. Pre-B cell receptor signaling induces immunoglobulin κ locus accessibility by functional redistribution of enhancer-mediated chromatin interactions. *PLoS Biol.* **12**, e1001791 (2014).
A report showing that signal-responsive transcription factors during B cell development can induce a functional redistribution of existing 3D enhancer–promoter interactions, a phenomenon called enhancer focusing.
141. Noordermeer, D. et al. Variegated gene expression caused by cell-specific long-range DNA interactions. *Nat. Cell Biol.* **13**, 944–951 (2011).
142. Gröschel, S. et al. A single oncogenic enhancer rearrangement causes concomitant *EVI1* and *GATA2* deregulation in leukemia. *Cell* **157**, 369–381 (2014).
A study showing that disrupted TAD organization can have major consequences for cancer development: chromosomal abnormalities enable a GATA2 enhancer to ectopically activate the *EVI1* oncogene while silencing GATA2, together resulting in cellular transformation.
143. Laurenti, E. & Göttgens, B. From haematopoietic stem cells to complex differentiation landscapes. *Nature* **553**, 418–426 (2018).
144. Wang, H. et al. CRISPR-mediated programmable 3D genome positioning and nuclear organization. *Cell* **175**, 1405–1417 (2018).
145. Barrington, C., Finn, R. & Hadjur, S. Cohesin biology meets the loop extrusion model. *Chromosome Res.* **25**, 51–60 (2017).
146. Ke, Y. et al. Chromatin structures of mature gametes and structural reprogramming during mammalian embryogenesis *Cell* **170**, 367–381 (2017).
147. Du, Z. et al. Allelic reprogramming of 3D chromatin architecture during early mammalian development. *Nature* **547**, 232–235 (2017).
148. Gassler, J. et al. A mechanism of cohesin-dependent loop extrusion organizes zygotic genome architecture. *EMBO J.* **36**, 3600–3618 (2017).
149. Jung, Y. H. et al. Chromatin states in mouse sperm correlate with embryonic and adult regulatory landscapes. *Cell Reports* **18**, 1366–1382 (2017).
150. Kaaij, L. J. T., van der Weide, R. H., Ketting, R. F. & de Wit, E. Systemic loss and gain of chromatin architecture throughout zebrafish development. *Cell Reports* **24**, 1–10 (2018).
151. Meshorer, E. et al. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev. Cell* **10**, 105–116 (2006).

Acknowledgements We apologize to the authors of many relevant studies for not citing their work owing to space limitations. We thank members of the Stadhouders, Filion and Graf laboratories for helpful discussions. G.F. and T.G. are supported by the European Research Council under the 7th Framework Programme FP7/2007-2013 (ERC Synergy Grant 4D-Genome, grant agreement 609989). R.S. is supported by the Netherlands Organization for Scientific Research (VENI 91617114) and an Erasmus MC Fellowship.

Reviewer information *Nature* thanks Peter Fraser, Konrad Hochedlinger and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions R.S. and T.G. conceived and wrote the manuscript with essential input from G.J.F. Figures were prepared by R.S.

Competing interests The authors declare no competing interests.

Additional information
Reprints and permissions information is available at http://www.nature.com/reprints.
Correspondence and requests for materials should be addressed to R.S. or T.G.
Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.