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Two major mechanisms of chromosome organization

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The spatial organization of chromosomes has long been connected to their polymeric nature and is believed to be important for their biological functions, including the control of interactions between genomic elements, the maintenance of genetic information, and the compaction and safe transfer of chromosomes to cellular progeny. chromosome conformation capture techniques, particularly Hi-C, have provided a comprehensive picture of spatial chromosome organization and revealed new features and elements of chromosome folding. Furthermore, recent advances in microscopy have made it possible to obtain distance maps for extensive regions of chromosomes (Bintu *et al.*, 2018; Nir *et al.*, 2018 [2••,3]), providing information complementary to, and in excellent agreement with, Hi-C maps. Not only has the resolution of both techniques advanced significantly, but new perturbation data generated in the last two years have led to the identification of molecular mechanisms behind large-scale genome organization. Two major mechanisms that have been proposed to govern chromosome organization are (i) the active (ATP-dependent) process of loop extrusion by Structural Maintenance of Chromosomes (SMC) complexes, and (ii) the spatial compartmentalization of the genome, which is likely mediated by affinity interactions between heterochromatic regions (Falk et al., 2019 [76••]) rather than by ATP-dependent processes. Here, we review existing evidence that these two processes operate together to fold chromosomes in interphase and that loop extrusion alone drives mitotic compaction. We discuss possible implications of these mechanisms for chromosome function.

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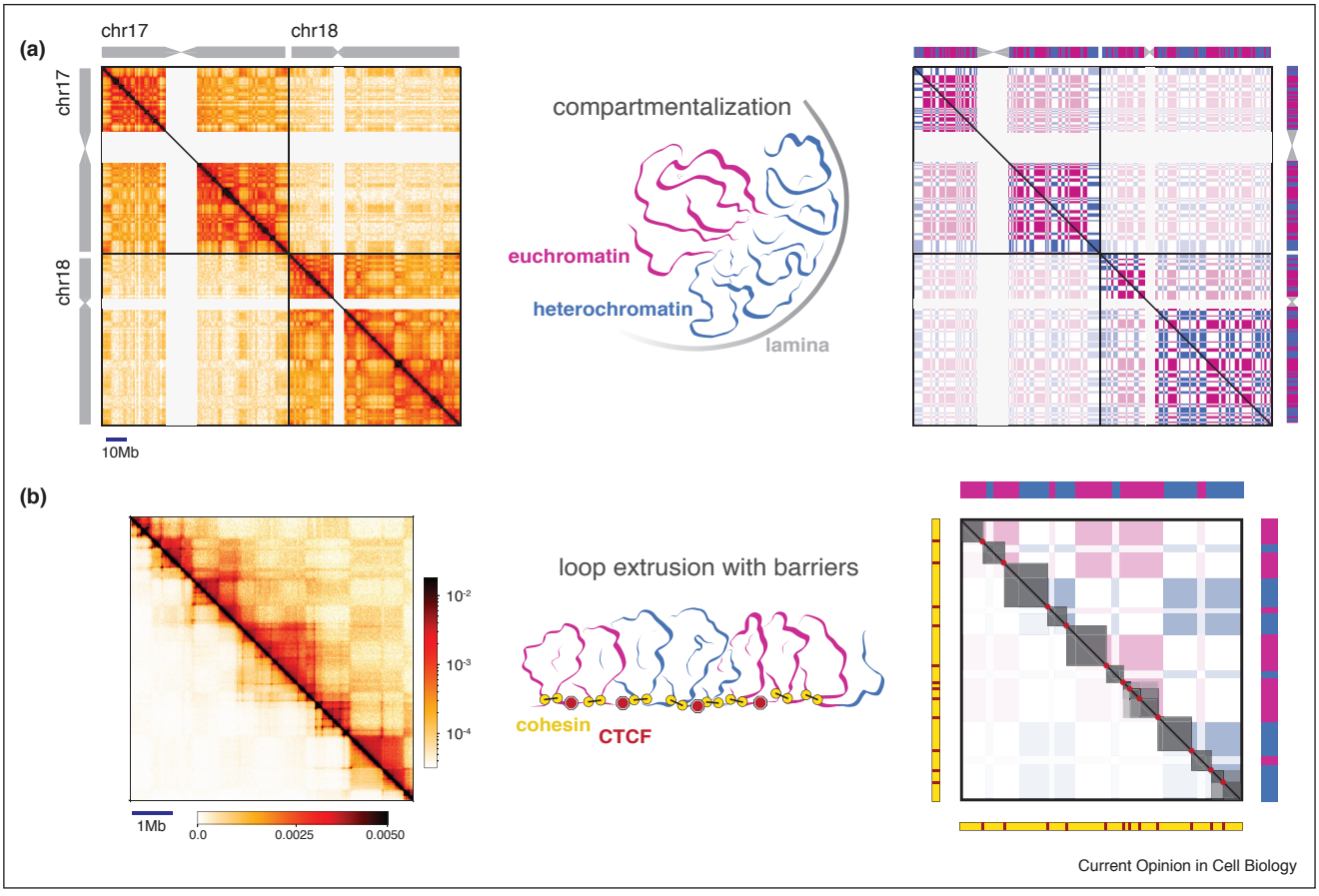
> "A map is not the territory it represents, but, if correct, it has a similar structure to the territory, which accounts for its usefulness." — Alfred Korzybski [4].

The map and the territory

Over the last two decades, chromosome conformation capture technologies, particularly Hi-C, have become the leading source of insights into chromosome organization. Patterns that appear in population Hi-C maps suggest different layers of chromosome organization; however, interpretation of these patterns requires careful consideration of what the data represent. Each DNA ligation event captured in a Hi-C experiment records a *contact* between two loci, meaning that in one cell, a pair of genomic fragments containing those two loci were in sufficient spatial proximity to ligate together. In most cases, these captured contacts are obtained in bulk from millions of cells to produce a *contact frequency map* (Hi-C map). Unlike the signal tracks from ChIP-seq, DNase-seq and similar methods, a contact frequency map cannot be reduced faithfully to a set of isolated peaks or features [5]; rather, it is a continuous signal with a variety of complex patterns occurring at different scales, and distinct biological processes can contribute to the formation of different patterns. By probing the effects of molecular perturbations on various classes of patterns, it is possible to distinguish the processes responsible for their formation and to elucidate their biophysical details.

Two major classes of patterns are evident in the contact frequency maps of higher eukaryotes (Figure 1). The first is the checkerboard-like pattern visible on Hi-C maps both within and between chromosomes [6] (Figure 1a). This pattern reflects the general phenomenon of active (euchromatin) and inactive (hetero)chromatin separating spatially in the nucleus [7], resulting in higher contact frequency between genomic regions having the same type and reduced contact frequency between regions of different type. This interpretation gives rise to the nomenclature of A/B compartments and *compartmentalization* to describe this pattern. Compartmentalization appears in all studied mammalian cell types with the exception of the mouse maternal pronucleus (Flyamer et al., 2017). The genomic intervals that roughly segment the contact map by compartmental type are variously termed compartments, compartmental regions, intervals, or domains in the literature [8]. In this review, we will refer to them as compartmental domains.

Figure 1



Hallmark patterns in Hi-C maps: compartmentalization and TADs.
(a) Compartmentalization. Pattern in Hi-C (left): The characteristic checkerboard pattern of alternating zones of contact enrichment and depletion spanning the intrachromosomal and interchromosomal areas of the genome-wide Hi-C map. Chromosomes 16 and 17 from HFFc6 human fibroblast cells are shown (data from https://data.4dnucleome.org). Interpretation (middle): Genomic intervals of different type exhibit differential affinities that cause them to segregate spatially in the nucleus. At the coarsest level, this can be broken down into two compartmental types: A (active, magenta) and B (inactive, blue) chromatin. Schematic (right): A simplified annotation, where colored rectangles represent zones of homotypic contact enrichment and blank rectangles represent zones of heterotypic contact depletion. The colors in the margin represent the A/B identity along the chromosomes. The translucence of *trans* and inter-arm areas of the map represents the different baseline contact frequencies associated with territoriality of chromosomes and chromosome arms as seen in Hi-C on the left.
(b) TADs and associated cohesin-dependent patterns. Pattern in Hi-C (left): Areas along the diagonal of the contact map at shorter genomic ranges (normally <3 Mb) show elevated contact frequencies and exhibit characteristic features such as stripes, peaks, and peak grids-based typically at CTCF sites. In this example region, the lower triangle of the heatmap uses a linear color scale that highlights the details of TAD patterns, while the upper triangle of the heatmap uses a logarithmic color scale that enables one to observe TAD patterns superimposed upon compartmental domains and their associated checkered zones. Interpretation (middle): An ATP-dependent process of loop extrusion by cohesin complexes locally compacts chromosomes and acts independently of the forces driving compartmentalization. Genomically localized barrier elements constrain the range by which loop extrusion can mediate contacts, which gives rise to TAD-associated patterns of contact enrichment [12•]. Schematic (right): A simplified depiction of the range of loop extrusion-mediated contact enrichment (dark grey squares) limited by barriers depicted in red on the diagonal and within the yellow bars in the margin. In the background, compartmental domains and zones of enrichment and depletion are colored as in (a). The lower triangle is translucent to reflect the dynamic range of signal when using a linear color scale as in the Hi-C example on the left.

The second set of patterns (Figure 1b), evident primarily in vertebrate maps, are cohesin-dependent and make up the features associated with Topologically Associating Domains (TADs). TADs are characterized as continuous regions where higher (around 2–4-fold) contact frequency is observed between loci inside each TAD than between loci in neighboring TADs [9,10]. Frequently, the edges of these square areas in Hi-C maps are enriched more than the interior, appearing as *stripes* (a.k.a. lines, tracks or 'flames', Figure 1b, right) [11••,12•]. Focal *peaks* of enriched contact frequency commonly appear as 'dots' at the outer corners of TAD squares or at the intersection of nearby TAD boundaries [5], visible most prominently in Micro-C maps (Krietenstein et al., 2019https://www.biorxiv.org/content/

10.1101/639922v1doi: 10.1101/639922(2); Hsieh et al., 2019https://www.biorxiv.org/content/10.1101/638775v1doi: 10.1101/638775). TAD-related patterns may also appear to exhibit several levels of nesting [13] in transitive configurations that can produce grid-like alignments of edges and peaks [11••,14,15]. Although sometimes used as a catchall term for any sort of contact 'domain', we will use the term TAD to refer to contiguous genomic regions exhibiting these cohesin-dependent patterns.

Although perturbation experiments have revealed that the processes underlying compartmental domains and TADs are biologically distinct (Schwarzer et al., 2017), it can be difficult to differentiate them, especially in lower resolution Hi-C maps, as both manifest square areas along the diagonal of Hi-C maps. Consequently, the two types of domain are very often conflated in the literature. There are, however, important differences between them: (i) Unlike compartmental domains, TADs do not exhibit long-range associations with other TADs other through an off-diagonal checkered pattern of contact frequency. (ii) All cohesin-dependent patterns are local, that is, formed by nearby (<3 Mb) genomic regions localized along the diagonal of Hi-C maps. By contrast, the compartmentalization checkerboard is a global pattern seen not only along the diagonal but also far from it, both within chromosomes and between chromosomes. These features help to distinguish compartments from TADs when analyzing Hi-C maps. Though they were originally thought to be organized hierarchically [16,17], it is now appreciated that genomic regions identifiable from Hi-C maps as TADs and compartmental domains span comparable length scales, overlap, and coexist throughout the genome in interphase.

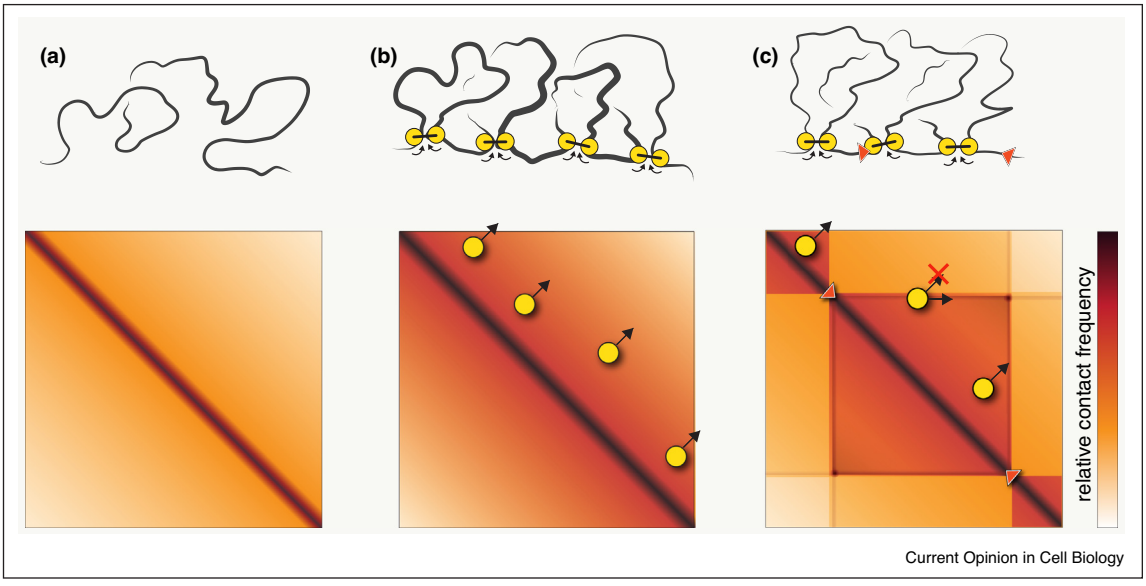
It is generally unfortunate that much of the established terminology to describe data features (e.g. domain, compartment) in Hi-C maps consists of suggestive terms that make it didactically difficult to differentiate a pattern on the map from its interpretation. In this review, we will refrain in particular from using the singular term 'loop' to describe a peak of locally elevated contact frequency (i) because any intrachromosomal contact may be thought of as a loop, (ii) because, to some, the term connotes a permanent tether, while the strength of a peak in a Hi-C map does not indicate the temporal stability of the underlying interactions [18•,19,20], and (iii) to avoid ambiguity when discussing underlying physical processes involving chromatin loop formation. We will use the word 'loop' exclusively in the physical and spatiotemporal sense, that is, as a stretch of chromatin fiber with two ends in close proximity.

Loop extrusion in interphase

Loop extrusion is a mechanism that has been conjectured several times in review articles since the 1990s [21–23], but it remained unexplored until 2012 [24]. In the loop extrusion process, a loop-extruding factor binds the chromatin fiber and reels it in from both sides, thus extruding a progressively growing loop. Once the loop-extruding factor dissociates, the loop is left to diffusively fall apart. The first theoretical studies [25] (11,26) demonstrated that this seemingly simple process when performed by multiple, independently acting, loop-extruding factors can reproduce a wealth of chromosomal phenomena such as the formation of chromosomal domains [11••] and the compaction and segregation of chromatids [25,26]. Knockdown experiments have convincingly demonstrated that proteins of the SMC family, cohesin and condensin, serve as loop-extruding factors [27••,28•,29,30•], and that during interphase, this activity is due entirely to cohesin and not condensin (Abdennur et al., 2019 [97]). Central for loop extrusion in interphase is the ability of certain DNA-bound proteins, such as CTCF, and possibly others (YY1 [31], Znf143 [32,33], etc.) to stop or pause loop-extruding cohesin complexes, thus serving as extrusion barriers [11••,14,34•]. In the case of CTCF, the barriers further appear to be *directional*, that is, they impede the traversal of complexes that extrude loops towards them from one side but not those approaching from the other. By interfering with loop extrusion, barrier-forming proteins can modulate the frequency at which different genomic elements are brought together. Simulations and experiments provide a wealth of evidence that this interplay between loop extrusion and barriers is what gives rise to TAD patterns in population Hi-C maps [12•].

There are two important mechanistic aspects as to how loop extrusion gives rise to TADs. First, TAD patterns arise not because loop extrusion prevents spontaneous spatial contacts between neighboring TADs, but because extruded loops mediate *additional* contacts within the regions that emerge as TADs (Figure 2). Because of the polymeric nature of chromatin, all loci on a chromosome are expected to exhibit baseline contact frequencies in the absence of extrusion. The process of loop extrusion compacts chromatin locally and the extruding loops produce additional contacts at short genomic ranges. An extrusion barrier (e.g. CTCF) blocks these extrusion-mediated contacts from propagating to pairs of loci on opposite sides of it, that is, between TADs. As a result, a TAD experiences an elevated number of contacts from within due to the extrusion of loops. Second, this elevated contact frequency cannot arise solely from two CTCF sites being tethered as a loop: it requires the continuous action and turnover of extruded loops mediating the additional contacts within the intervening DNA [11••,35]. In summary, loop extrusion increases contact frequency largely within TADs, making them appear 'insulated', but does little to prevent spatial contacts between neighboring TADs, in contrast to many cartoons where TADs appear as crumples of chromatin separated from each other.

Figure 2



TAD formation by loop extrusion. Bottom: diagrams of population contact frequency maps of a genomic region. **(a)** Basal level of contact frequency in the absence of loop extrusion. **(b)** Loop extrusion creates additional short-range contacts, further compacting chromatin. No extrusion-dependent domains are visible in Hi-C in the absence of barriers. **(c)** Extrusion barriers limit the additional extrusion-mediated contacts to intervals delimited by barriers, thus giving rise to TADs, while the contact frequency between TADs is lower. Hence, loop extrusion forms TADs by mediating additional intra-TAD contacts, rather than by insulating spontaneous spatial contacts. Stripes and peaks also naturally emerge when cohesins are stopped by CTCF at TAD borders. Top: cartoon depicting a single chromatin fiber (a) without loop extrusion; (b) subject to loop extrusion; (c) subject to loop extrusion with barriers. Symbols are drawn both on the cartoons to illustrate the extrusion machinery on chromatin and on top of the map to indicate the possible instantaneous positions of loop extruders in the example conformation above. Yellow circles depict loop extruders, and red triangles depict directional extrusion barriers. Black arrows on the map depict directions of cohesin translocation.

Extrusion barriers can also direct and target extrusion-mediated interactions between genomic loci. For example, a minimal loop extrusion model provides a compelling scenario that explains the formation of stripes at CTCF sites: an extruder having reached a single barrier in one direction can continue to track along the chromatin fiber in the other direction, connecting this site to downstream loci. A Hi-C map accumulates snapshots of this dynamic process over a population of cells, giving rise to a stripe of higher contact frequency emanating from the barrier site. Two proximal CTCF sites contact one another more frequently because both of them impede loop-extruding cohesins. When sampling snapshots from a population, contacts mediated by either a single extruded loop or any succession of consecutive loops (rosette) connecting two barriers can contribute to the corner peak intensity observed at the intersection of two TAD boundaries. Indeed, simulations of loop extrusion with directional and partially permeable barriers [11••,12•,14] can reproduce a broad range of features seen in Hi-C maps, including stripes along TAD edges, peaks at TAD corners between convergently oriented CTCFs and grids of peaks [5,36]. Furthermore, while these simulations assume unbiased loading and dissociation at all locations, they nevertheless predict high occupancy of loop extruding complexes at barrier sites. Importantly, the often-noted nested appearance of TADs in Hi-C maps arises without hierarchical spatial folding because extrusion barriers at CTCF-binding sites are both directional and vary in their degree of permeability (e.g. due to do different numbers of binding sites, levels of CTCF occupancy, and rates of CTCF exchange [37]).

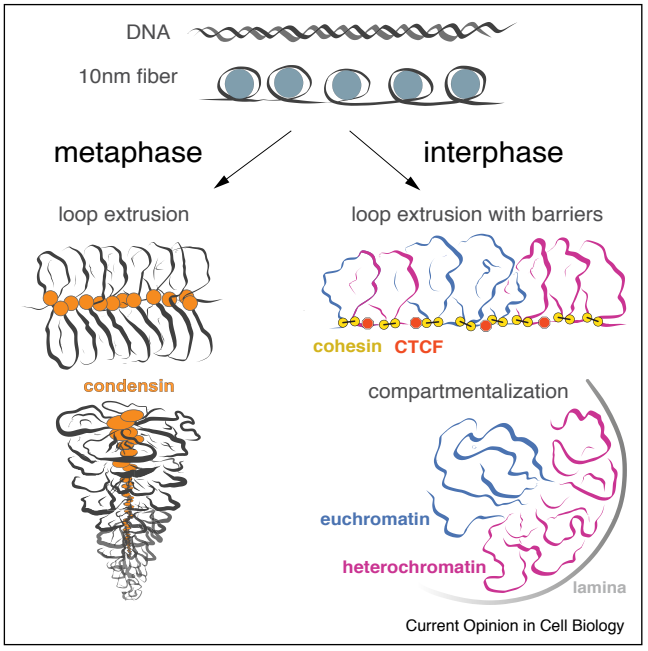
Other mechanisms proposed to explain formation of TADs have significant limitations. Some studies achieve insulation of domains from each other by making different TADs out of monomers of different types that have selective affinities for one another [38–40]. However, because affinities also drive attracted domains together and unattracted domains apart, they necessarily generate an off-diagonal checkerboard characteristic of compartmental domains and, therefore, these may be considered models of compartmentalization rather than TADs (see discussion on phase separation). Moreover, such affinity-based models cannot explain the experimentally observed fusion of neighboring TADs upon loss of CTCF or engineered deletion of CTCF sites [14,34•,41,42]. Another hypothesized mechanism of TAD formation is based on CTCF–CTCF affinity [16]. While CTCF–CTCF affinity can lead to formation of stable loops between TAD borders, such loops cannot lead to enrichment of contact frequency within TADs, as explained above. Moreover,

affinity between CTCF sites would lead to elevated contact frequency between every pair of CTCF-occupied loci in all orientations, including pairs at large genomic distances and between chromosomes. Hi-C data, on the contrary, does not show enrichment for pairs of CTCF sites separated by more than 3 Mb. Finally, models in which TAD borders have different physical properties of chromatin (rigidity, thickness, etc.) [16] have been studied by simulations but failed to provide relative insulation of neighboring TADs at length-scales larger than the sizes of borders [11••].

The loop extrusion mechanism is currently supported by a wealth of evidence based on Hi-C [27••,28•,29,30•,34•,43•], multi-point-4C [44], microscopy [2••] and single-molecule experiments [45••,46••] (see Ref. [12•] for review). The predictions of the loop extrusion model agree with the experiments where (i) CTCF was depleted globally [30•,34•], or by deleting/modifying CTCF sites [9,14,42,47,48]; (ii) chromatin-associated cohesin or all cohesin were depleted [27••,28•,29,30•] or enriched [29,30•,43•]. Moreover, recent studies [49••] have convincingly demonstrated that loop extrusion is ATP-dependent and transcription-independent, though association and dissociation of cohesin could be coupled with transcription [50] and other genomic processes. The agreement between the loop-extrusion model and knockdown experiments goes beyond the loss of TADs, predicting decompaction upon cohesin loss, but not upon loss of CTCF—these effects are seen in Hi-C data (see Ref. [12•] for review) and decompaction upon cohesin loss has also been observed via microscopy [2••]. Another observation of loop extrusion comes from bacteria, *Bacillus subtilis* and *Caulobacter crescentus*, where SMCs zip two chromosomal arms together [51••,52•] allowing direct *in vivo* visualization of progressive loop extrusion and measurement of its speed in bacteria (25–50 kb/min). Single-molecule studies have clearly demonstrated that SMC complexes have ATP-dependent DNA-translocation [45••,53] and loop extrusion [46••] activity on naked DNA, and can overcome nucleosomes but not CTCF while sliding [54]. Directly observing the process of loop extrusion *in vivo* remains a daunting challenge as extrusion-mediated contacts are very dynamic (SMCs exchange every 5–20 min and move at speeds ~10–50 kb/min) and may not appear different from other spatial contacts. Since the chromatin anchor points of an extruding loop at any given time in any given cell are not special, neither single-cell Hi-C nor superresolution microscopy can tell them apart from myriad other physical contacts.

The molecular mechanism of loop extrusion by SMCs is unknown and is being actively investigated ([45••,53,55,56]; reviewed in Ref. [57]). One open question is whether loop extrusion proceeds in both directions from the site of SMC association as predicted by the models [11••,14], or whether it reels in DNA only on one side as seen *in vitro* for yeast condensin in single-molecule

Figure 3



Two major mechanisms of chromosome organization. Nucleosomal arrays create a chromatin fiber that is folded differently in interphase and metaphase. In mitosis, loop extrusion by condensins leads to the formation of a dense array of loops: condensins form a scaffold with loop emanating from it. In interphase, loop extrusion is performed by cohesin forming a sparse dynamic array of loops, with CTCFs forming barriers to extrusion. Heterochromatin and euchromatin are simultaneously compartmentalized in space by the attraction of heterochromatic regions to one another.

experiments [46••]. A recent theoretical study [58] suggested that effective chromosome compaction (e.g. ~1000-fold for vertebrate chromosomes) requires two-sided extrusion, stimulating the search for effectively two-sided extrusion performed by vertebrate SMC complexes either solo or in higher-order complexes. Other open questions include the mechanism that allows CTCF to interact with cohesin and impede extrusion in spite of the relatively rapid turnover of CTCF on DNA [37]; the impact of targeted cohesin loading and unloading; interactions of extrusion with transcription [59], as well as other possible mechanisms of regulation of the extrusion-with-barriers system.

Loop extrusion for chromosome compaction and gene regulation

Loop extrusion has been historically [21–23] and recently [24–26,60] implicated in chromosome compaction (Figure 3). Theory and simulations have demonstrated that exchanging loop-extruding factors can fold a chromosome into a gapless array of consecutive loops, achieving ~1000-fold lengthwise compaction. In 3D, such loop arrays acquire a 'bottle-brush' shape, with loops emanating from a central scaffold formed by loop-extruders. This organization was shown to be

consistent with Hi-C for mitotic chromosomes [60,61••]. Moreover, supplemented by topoisomerase II activity, loop extrusion leads to segregation and disentanglement of sister chromatids. A recent Hi-C study performed across different time points through prophase and prometaphase probed the dynamics of chromosome compaction [61••]. By analyzing the time-resolved Hi-C, it was shown that gradual mitotic compaction can be explained by processes achievable through extrusion: progressive enlargement of consecutive loops and the formation of nested loops. Indeed, knockdowns of condensin II and condensin I support their roles in formation of consecutive and nested loops, respectively, in mitotic chromosomes [61••].

TADs have long been implicated in the regulation of gene expression. Surprisingly, experiments removing cohesin and CTCF from chromosomes showed limited effects on steady-state transcription [27••,28•,34•]. However, more recently, it was shown that cohesin is necessary for the expression of inducible genes and the activation of inducible enhancers in macrophages and myeloid progenitor cells triggered by inflammatory stimuli [62]. This role of cohesin is consistent with earlier works that show that reduced levels of cohesin promote self-renewal and impair cell differentiation [63,64]. A striking example of the role of the cohesin-CTCF system is enhancer–promoter communication in the protocadherin gene cluster, whose exons are expressed combinatorially to generate a diverse cell surface identity code [65,66]. These results suggest that cohesin-dependent loop extrusion may play a critical role in cellular decision making (e.g. establishing transcriptional states during differentiation and in response to stimuli), while possibly having a limited impact on steady-state transcription. Future studies probing temporal stimulus-response dynamics will hopefully shed more light on the relationship between loop extrusion and gene regulation.

Compartmental segregation by phase separation

The spatial separation of transcriptionally active euchromatin and largely silent heterochromatin is a prominent hallmark of chromosome organization seen in interphase nuclei in both Hi-C and microscopy [see Ref. [7] for review] (Figure 1). In the conventional picture, the center of the nucleus is occupied by euchromatin, while peripheral, nucleolus-associated and chromocenter-associated areas are occupied by heterochromatin—both constitutively silent and facultatively repressed. This segregation is seen in population Hi-C maps as a checkered pattern of interaction enriched between regions of the same chromatin type, and depleted between regions of different type. The pattern extends not only throughout each chromosome but between all chromosomes as well (Figure 1).

In contrast to TADs which are associated with sharp border elements, compartmental domains appear to be defined by the chromatin state of their interior [67] and never exhibit a nested appearance in the absence of TADs. While the exact molecular players that drive compartmental organization are not yet known, cohesin/CTCF can be ruled out since removal of cohesin leads to enhanced compartmentalization patterns in Hi-C [27••,28•,29,30•] and removal of CTCF leaves them unperturbed [34•]. Moreover, mouse maternal pronuclei Hi-C maps exhibit TAD patterns but lack those of compartmentalization (Flyamer et al., 2017). Epigenetic signatures such as histone marks and transcriptional state can be used to predict the compartmental segmentation in fly and mammalian cells [38,39,67–69]. However, the relationships between compartmentalization, gene expression, the deposition, and maintenance of epigenetic marks, and the recruitment of transcription factors remain to be disentangled. For example, it is proposed that positive feedback between spatial coalescence of like chromatin and genomic bookmarks can sustain the memory of epigenetic domains across cell divisions [70,71].

From the physics point of view, this spatial segregation of unlike chromatin is reminiscent of microphase separation in polymers with two (or more) types of monomers (a system referred to as a *block copolymer*) [39,68,72,73]. Even weak attraction between monomers of the same type is sufficient to drive phase separation in polymer systems. Hence, attraction among euchromatic regions, or among heterochromatic ones, can be possible mechanisms of compartmentalization. Additionally, the localization of heterochromatin next to the nuclear lamina suggests that the attraction between the lamina and heterochromatin could play a role in compartmentalization. Recent studies have aimed to disentangle the roles of the lamina from interactions between chromatin by using information about nuclear organization in cells missing some components of the lamina: progeria cells [74,75] or cells with naturally or artificially 'inverted nuclei' [76••]. The surprising answer is that heterochromatin–lamina interactions are dispensable for compartmental segregation. The phenomenon of nuclear inversion, that is, re-localization of heterochromatin to the center and euchromatin to nuclear periphery upon loss of interactions with the lamina, strongly suggests that affinity of heterochromatic regions for one another is the primary driver of compartmentalization, while interactions with the lamina are important for the peripheral positioning of heterochromatin. The processes of compartmentalization, however, are very slow as they involve all nuclear chromatin [77], and, in rapidly dividing cells, some aspects of this global organization can be in part determined by the initial positions of chromosomes, nucleoli and their interactions with the lamina upon exit from mitosis [78].

Taken together, recent studies have revealed two major mechanisms of chromosome organization during interphase: (i) the heterochromatin-driven compartmentalization of chromatin that is reminiscent of microphase separation; and (ii) the active (ATP-dependent) loop extrusion that can be controlled by barriers at specific DNA positions and can in turn facilitate and control interactions between genomic elements (Figure 3). The interplay between these processes has also been recently observed experimentally and studied theoretically. Interestingly, active loop extrusion interferes with compartmental separation [27••,79]. When chromatin-associated cohesin is depleted, (a) compartmentalization patterns gets stronger and (b) shorter compartmental domains become more prominent in Hi-C [27••]. Consistently, an increase in cohesin activity (in Wapl knockout cells) leads to weakened compartmentalization signal [29,30•,43•]. Simulations show that this is a general polymer physics phenomenon where the active process of loop extrusion (irrespective of extrusion barriers) antagonizes the proper phase separation of a block copolymer. Thus, loop extrusion weakens global compartmentalization by locally mixing compartmental domains of different type, having a particularly strong impact on short (<100 Kb) euchromatic or heterochromatin regions. While this highlights the impact of loop extrusion on compartmentalization of regions with different epigenetic states, the influence of epigenetic state on loop extrusion dynamics remains to be uncovered.

Other emerging mechanisms

Other processes shape genome organization besides loop extrusion and compartmentalization. For example, the pairing of homologous chromosomes recently visualized at the whole-genome level by Hi-C in fly embryos [80] and cell lines [81] shows a variety of new patterns that exist alongside compartmentalization and are likely formed by another mechanism. The pairing of sister chromatids recently studied by live-cell microscopy [82] is also driven by cohesin [83,84], but possibly through a non-extrusion mechanism.

The process of transcription can also play a role in pausing loop extrusion and/or relocalizing cohesin [54,85], thereby influencing chromosome organization. This effect is seen most clearly in bacteria [52•,59] where loop extrusion slows down at highly transcribed rRNA genes. These effects can also play a role in eukaryotic chromosome organization [86].

Affinity between specific genomic elements is emerging as an independent mechanism of chromosome folding. Such affinity can be detected in Hi-C as an enrichment of observed contact frequency over the baseline established by the compartmentalization and extrusion-mediated patterns. For example, recent studies suggested that highly transcribed, exon-rich genes interact more frequently, likely due to co-localization at splicing factories [87•,88,89]. Olfactory receptors interact more frequently with each other in *cis* and in *trans* than would be expected from compartmentalization [90], suggesting affinity-based mechanism mediated by specific proteins. Polycomb-silenced loci are typically located within euchromatin and show a pattern of interactions elevating above other signals and suggesting affinity-based clustering [91]. It remains to be seen whether other genomic elements, for example, superenhancers and their targets, interact more frequently with, and hence have greater affinity for one another than would be expected from loop extrusion-mediated patterns and compartmentalization [92].

Hi-C and microscopy: complementary and consistent pictures

Hi-C and microscopy provide largely complementary views of genome organization. Hi-C captures events of proximity between loci, while microscopy measures the whole distribution of distances between loci, potentially providing more information [18•]. While historically microscopy was limited to interrogating only a limited set of loci, recent studies [2••,3] have scaled up FISH microscopy by tiling 1–5 MB regions with unique probes, tracing continuous genomic regions with a resolution of 30 kb.

Microscopy using both localized and tiling probes agree with Hi-C data. First, contact frequency measured by Hi-C is strongly anticorrelated with the median distance between loci measured by microscopy [19,93,94], though the nature of the observed relationship between the two remains unknown. Second, both compartments and TADs have been observed in microscopy: the former was manifested as lower distances between pairs of regions of the same compartment type comparing those of different type [93]; the latter as lower distances between locus pairs within the same TADs compared to those in different TADs [2••,3,19]. Third, microscopy has confirmed that the loss of cohesin leads to disappearance of TADs. Uniquely, microscopy also showed an increase in intra-TAD distances upon cohesin loss, directly demonstrating the role of cohesin in local compaction of chromatin [2••].

Single-cell conformations captured by microscopy [2••] resemble the contact organization seen in single-cell Hi-C [29,95] in that both methods have measured enrichment of contacts or lower distances within TADs despite widespread 'violations' of TAD borders in individual cells and 'clumpiness' of contacts (both with and without cohesin). While it may seem paradoxical on the surface, these results actually come as no surprise. First, the apparently 'illegal' contacts between TADs reflect baseline contact frequencies that naturally occur since, as discussed earlier, loop extrusion does not act to prevent

spontaneous association of loci in neighboring TADs, but rather mediates additional contacts within TADs. Second, clumps of contacts observed with or without loop extrusion reflect the tendency of neighboring regions of a polymer to spontaneously cluster together. Similar violations and clumpiness are observed in individual snapshots from simulations with and without loop extrusion [11••], strongly suggesting that their origin lies in polymeric effects and the nature of domain formation via loop extrusion.

Conclusions

Two independent processes shape chromosome organization: affinity-driven compartmentalization and active loop extrusion. Loop extrusion by SMC complexes is an ATP-dependent process that can compact chromosomes during mitosis, and when constrained by barriers during interphase, can generate TADs and other local features of chromosome organization. Proposed on the basis of theoretical considerations [11••], loop extrusion is now supported by extensive experimental evidence [27••,28•,29,30•,34•,43•,45••,46••,51••,61••]. Compartmentalization appears to be driven predominantly by interactions between heterochromatic regions [76••]. The molecular players are still uncertain, but the process likely depends on histone marks that directly or indirectly interact with one another. The spatial positioning of compartments is determined by the anchoring of heterochromatin to the nuclear lamina [96]. Alongside these two major mechanisms, additional processes (pairing of homologs and sisters [80,81]), transcription [52•,59,86] and affinities between specific genomic regions mediated by specific molecular complexes (polycomb [91], splicing factors [89], etc.) shape global chromosome organization. The nature of these biophysical processes and their underpinning molecular mechanisms remain to be unraveled.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

2. •• Bintu B, Mateo LJ, Su J-H, Sinnott-Armstrong NA, Parker M, Kinrot S, Yamaya K, Boettiger AN, Zhuang X: **Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells**. *Science* 2018, **362**.
Distance maps obtained by microscopy show small distance for loci within, and larger between, TADs. Upon cohesin depletion, intra-TAD distances go up. This decompaction upon loss of cohesin is consistent with loop extrusion and was proposed in Fudenberg *et al.* [11••].

3. Nir G, Farabella I, Pérez Estrada C, Ebeling CG, Beliveau BJ, Sasaki HM, Lee SD, Nguyen SC, McCole RB, Chattoraj S *et al.*: **Walking along chromosomes with super-resolution imaging, contact maps, and integrative modeling**. *PLoS Genet* 2018, **14**: e1007872.

4. Korzybski A: *Science and Sanity: An Introduction to Non-Aristotelian Systems and General Semantics*. The International Non-Aristotelian Library Pub. Co.; 1933.

5. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping**. *Cell* 2014, **159**:1665-1680.

6. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO *et al.*: **Comprehensive mapping of long-range interactions reveals folding principles of the human genome**. *Science* 2009, **326**:289-293.

7. Solovei I, Thanisch K, Feodorova Y: **How to rule the nucleus: divide et impera**. *Curr Opin Cell Biol* 2016, **40**:47-59.

8. Rowley MJ, Corces VG: **Organizational principles of 3D genome architecture**. *Nat Rev Genet* 2018, **19**:789-800.

9. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J *et al.*: **Spatial partitioning of the regulatory landscape of the X-inactivation centre**. *Nature* 2012, **485**:381-385.

10. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B: **Topological domains in mammalian genomes identified by analysis of chromatin interactions**. *Nature* 2012, **485**:376-380.

11. •• Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA: **Formation of chromosomal domains by loop extrusion**. *Cell Rep* 2016, **15**:2038-2049.
This paper put forward hypothesis that loop extrusion with barriers underlies the formation of TADs during interphase. Using simulations, it was shown that loop extrusion with barriers quantitatively reproduces not only TADs, but also other patterns observed in Hi-C, including CTCF-CTCF contact peaks, and stripes; made major predictions of effects of CTCF and cohesin depletions.

12. • Fudenberg G, Abdennur N, Imakaev M, Goloborodko A, Mirny LA: **Emerging evidence of chromosome folding by loop extrusion**. *Cold Spring Harb Symp Quant Biol* 2017, **82**:45-55.
An important review that compares predictions of the loop extrusion theory with the experimental outcomes of cohesin, CTCF, and Wapl depletion.

13. Phillips-Cremins JE, Sauria MEG, Sanyal A, Gerasimova TI, Lajoie BR, Bell JSK, Ong C-T, Hookway TA, Guo C, Sun Y *et al.*: **Architectural protein subclasses shape 3D organization of genomes during lineage commitment**. *Cell* 2013, **153**:1281-1295.

14. Sanborn AL, Rao SSP, Huang S-C, Durand NC, Huntley MH, Jewett AI, Bochkov ID, Chinnappan D, Cutkosky A, Li J *et al.*: **Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes**. *Proc Natl Acad Sci U S A* 2015, **112**:E6456-E6465.

15. Mumbach MR, Satpathy AT, Boyle EA, Dai C, Gowen BG, Cho SW, Nguyen ML, Rubin AJ, Granja JM, Kazane KR *et al.*: **Enhancer connectome in primary human cells identifies target genes of disease-associated DNA elements**. *Nat Genet* 2017, **49**:1602-1612.

16. Dixon JR, Gorkin DU, Ren B: **Chromatin domains: the unit of chromosome organization**. *Mol Cell* 2016, **62**:668-680.

17. Gibcus JH, Dekker J: **The hierarchy of the 3D genome**. *Mol Cell* 2013, **49**:773-782.

18. • Fudenberg G, Imakaev M: **FISH-ing for captured contacts: towards reconciling FISH and 3C**. *Nat Methods* 2017, **14**:673-678.
This study describes how complementary information can be obtained by microscopy and Hi-C.

19. Finn EH, Pegoraro G, Brandão HB, Valton A-L, Oomen ME, Dekker J, Mirny L, Misteli T: **Extensive heterogeneity and intrinsic variation in spatial genome organization**. *Cell* 2019, **176**:1502-1515.e10.

20. Cattoni DI, Cardozo Gizzi AM, Georgieva M, Di Stefano M, Valeri A, Chamousset D, Houbron C, Déjardin S, Fiche J-B, González I *et al.*: **Single-cell absolute contact probability detection reveals chromosomes are organized by multiple low-frequency yet specific interactions**. *Nat Commun* 2017, **8**:1753.

21. Riggs AD, Holliday R, Monk M, Pugh JE: **DNA methylation and late replication probably aid cell memory, and type I DNA reeling could aid chromosome folding and enhancer function**. *Philos Trans R Soc Lond B Biol Sci* 1990, **326** http://dx.doi.org/10.1098/rstb.1990.0012.

22. Kimura K, Rybenkov VV, Crisona NJ, Hirano T, Cozzarelli NR: **13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation**. *Cell* 1999, **98**:239-248.

23. Nasmyth K: **Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis**. *Annu Rev Genet* 2001, **35**:673-745.

24. Alipour E, Marko JF: **Self-organization of domain structures by DNA-loop-extruding enzymes**. *Nucleic Acids Res* 2012, **40**:11202-11212.

25. Goloborodko A, Marko JF, Mirny LA: **Chromosome compaction by active loop extrusion**. *Biophys J* 2016, **110**:2162-2168.

26. Goloborodko A, Imakaev MV, Marko JF, Mirny L: **Compaction and segregation of sister chromatids via active loop extrusion**. *eLife* 2016, **5**.

27. •• Schwarzer W, Abdennur N, Goloborodko A, Pekowska A, Fudenberg G, Loe-Mie Y, Fonseca NA, Huber W, H Haering C, Mirny L *et al.*: **Two independent modes of chromatin organization revealed by cohesin removal**. *Nature* 2017, **551**:51-56.

This is the first successful depletion of chromatin-associated cohesin that resulted in complete loss of TADs and other cohesin-associated Hi-C patterns. Moreover, the study reported that upon cohesin depletion compartmentalization got stronger and finer, suggesting two separate mechanisms discussed here.

28. • Rao SSP, Huang S-C, Glenn St Hilaire B, Engreitz JM, Perez EM, Kieffer-Kwon K-R, Sanborn AL, Johnstone SE, Bascom GD, Bochkov ID *et al.*: **Cohesin loss eliminates all loop domains**. *Cell* 2017, **171**:305-320.e24.

This paper presents experiments where cohesin was acutely degraded, leading to the loss of TADs and peaks, which reappear once cohesin was restored. These experiments demonstrate direct role of cohesin in establishing these features.

29. Gassler J, Brandão HB, Imakaev M, Flyamer IM, Ladstätter S, Bickmore WA, Peters J-M, Mirny LA, Tachibana K: **A mechanism of cohesin-dependent loop extrusion organizes zygotic genome architecture**. *EMBO J* 2017, **36**:3600-3618.

30. • Wutz G, Várnai C, Nagasaka K, Cisneros DA, Stocsits RR, Tang W, Schoenfelder S, Jessberger G, Muhar M, Hossain MJ *et al.*: **Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins**. *EMBO J* 2017, **36**:3573-3599.

The experiments in this study provide a comprehensive demonstration that modulation of the levels of chromatin-associated cohesin and CTCF affects TAD-associated patterns in Hi-C maps.

31. Weintraub AS, Li CH, Zamudio AV, Sigova AA, Hannett NM, Day DS, Abraham BJ, Cohen MA, Nabet B, Buckley DL *et al.*: **YY1 is a structural regulator of enhancer-promoter loops**. *Cell* 2017, **171**:1573-1588.e28.

32. Bailey SD, Zhang X, Desai K, Aid M, Corradin O, Cowper-Sal Lari R, Akhtar-Zaidi B, Scacheri PC, Haibe-Kains B, Lupien M: **ZNF143 provides sequence specificity to secure chromatin interactions at gene promoters**. *Nat Commun* 2015, **2**:6186.

33. Wen Z, Huang Z-T, Zhang R, Peng C: **ZNF143 is a regulator of chromatin loop**. *Cell Biol Toxicol* 2018, **34**:471-478.

34. • Nora EP, Goloborodko A, Valton A-L, Gibcus JH, Uebersohn A, Abdennur N, Dekker J, Mirny LA, Bruneau BG: **Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization**. *Cell* 2017, **169**:930-944.e22.

This study shows that CTCF depletion leads to genome-wide fusion of TADs, consistent with the instructional role of CTCF in establishing TAD boundaries by serving as extrusion barriers.

35. Doyle B, Fudenberg G, Imakaev M, Mirny LA: **Chromatin loops as allosteric modulators of enhancer-promoter interactions**. *PLoS Comput Biol* 2014, **10**:e1003867.

36. Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odom DT, Tanay A, Hadjur S: **Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture**. *Cell Rep* 2015, **10**:1297-1309.

37. Hansen AS, Pustova I, Cattoglio Claudia, Tjian Robert, Darzacq X: **CTCF and cohesin regulate chromatin loop stability with distinct dynamics**. *eLife* 2017, **6**:e25776.

38. Barbieri M, Chotalia M, Fraser J, Lavitas L-M, Dostie J, Pombo A, Nicodemi M: **Complexity of chromatin folding is captured by the strings and binders switch model**. *Proc Natl Acad Sci U S A* 2012, **109**:16173-16178.

39. Jost D, Carrivain P, Cavalli G, Vaillant C: **Modeling epigenome folding: formation and dynamics of topologically associated chromatin domains**. *Nucleic Acids Res* 2014, **42**:9553-9561.

40. Chiariello AM, Annunziatella C, Bianco S, Esposito A, Nicodemi M: **Polymer physics of chromosome large-scale 3D organisation**. *Sci Rep* 2016, **6**:29775.

41. de Wit E, Vos ESM, Holwerda SJB, Valdes-Quezada C, Verstegen MJAM, Teunissen H, Splinter E, Wijchers PJ, Krijger PHL, de Laat W: **CTCF binding polarity determines chromatin looping**. *Mol Cell* 2015, **60**:676-684.

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

43. • Haarhuis JHI, van der Weide RH, Blomen VA, Yáñez-Cuna JO, Amendola M, van Ruiten MS, Krijger PHL, Teunissen H, Medema RH, van Steensel B *et al.*: **The cohesin release factor WAPL restricts chromatin loop extension**. *Cell* 2017, **169**:693-707.e14.

This study was the first to demonstrate that reducing chromatin-associated cohesin leads to weakening of TADs, while its enrichment enhances TADs, stripes and peaks.

44. Allahyar A, Vermeulen C, Bouwman BAM, Krijger PHL, Verstegen MJAM, Geeven G, van Kranenburg M, Pieterse M, Straver R, Haarhuis JHI *et al.*: **Enhancer hubs and loop collisions identified from single-allele topologies**. *Nat Genet* 2018, **50**:1151-1160.

45. •• Terakawa T, Bisht S, Eeftens JM, Dekker C, Haering CH, Greene EC: **The condensin complex is a mechanochemical motor that translocates along DNA**. *Science* 2017, **11**.

The first single-molecule study to demonstrate ATP-dependent motor activity by SMCs.

46. •• Ganji M, Shaltiel IA, Bisht S, Kim E, Kalichava A, Haering CH, Dekker C: **Real-time imaging of DNA loop extrusion by condensin**. *Science* 2018, **360**:102-105.

The first single-molecule study to directly observe and characterize active loop extrusion by SMCs.

47. Narendra V, Rocha PP, An D, Raviram R, Skok JA, Mazzoni EO, Reinberg D: **CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation**. *Science* 2015, **347**:1017-1021.

48. Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R *et al.*: **Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions**. *Cell* 2015, **161**:1012-1025.

49. •• Vian L, Pe?kowska A, Rao SSP, Kieffer-Kwon K-R, Jung S, Baranello L, Huang S-C, El Khattabi L, Dose M, Pruett N *et al.*: **The energetics and physiological impact of cohesin extrusion**. *Cell* 2018, **175**:292-294.

This study shows that establishing cohesin-associated patterns requires ATPase activity, thus strongly supporting ATP-dependent loop extrusion.

50. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS *et al.*: **Mediator and cohesin connect gene expression and chromatin architecture**. *Nature* 2010, **467**:430-435.

51. •• Wang X, Brandao HB, Le TBK, Laub MT, Rudner DZ: ***Bacillus subtilis* SMC complexes juxtapose chromosome arms as they travel from origin to terminus**. *Science* 2017, **355**:524.
Time-resolved Hi-C upon induction of SMC loading in bacteria allows to monitor SMC-mediated loop extrusion which zips chromosomal arms together. This study demonstrated progressive zipping, consistent with progressive loop extrusion at the speed of 50 Kb/min.

52. • Tran NT, Laub MT, Le TBK: **SMC progressively aligns chromosomal arms in *Caulobacter crescentus* but is antagonized by convergent transcription**. *Cell Rep* 2017, **20**:2057-2071.
Progressive zipping of chromosomal arms in Caulobacter shows loop extrusion by bacterial SMCs *in vivo*. This study also demonstrates that loop extrusion can be slowed down by convergent transcription.

53. Eeftens JM, Bisht S, Kerssemakers J, Kschonsak M, Haering CH, Dekker C: **Real-time detection of condensin-driven DNA compaction reveals a multistep binding mechanism**. *EMBO J* 2017, **36**:3448-3457.

54. Davidson IF, Goetz D, Zaczek MP, Molodtsov MI, Huis In't Veld PJ, Weissmann F, Litos G, Cisneros DA, Ocampo-Hafalla M, Ladurner R *et al.*: **Rapid movement and transcriptional re-localization of human cohesin on DNA**. *EMBO J* 2016, **35**:2671-2685.

55. Marko JF, De Los Rios P, Barducci A, Gruber S: **DNA-segment-capture model for loop extrusion by structural maintenance of chromosome (SMC) protein complexes**. *bioRxiv* 2018 http://dx.doi.org/10.1101/325373.

56. Diebold-Durand M-L, Lee H, Ruiz Avila LB, Noh H, Shin H-C, Im H, Bock FP, Bürmann F, Durand A, Basfeld A *et al.*: **Structure of full-length SMC and rearrangements required for chromosome organization**. *Mol Cell* 2017, **67**:334-347.e5.

57. Nichols MH, Corces VG: **A tethered-inchworm model of SMC DNA translocation**. *Nat Struct Mol Biol* 2018, **25**:906-910.

58. Banigan EJ, Mirny LA: **Limits of chromosome compaction by loop-extruding motors**. *bioRxiv* 2018 http://dx.doi.org/10.1101/476424.

59. Brandão HB, Wang X, Paul P, van den Berg AA, Rudner DZ, Mirny LA: **RNA polymerases as moving barriers to condensin loop extrusion**. *bioRxiv* 2019 http://dx.doi.org/10.1101/604280.

60. Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA, Dekker J: **Organization of the mitotic chromosome**. *Science* 2013, **342**:948-953.

61. •• Gibcus JH, Samejima K, Goloborodko A, Samejima I, Naumova N, Nuebler J, Kanemaki MT, Xie L, Paulson JR, Earnshaw WC *et al.*: **A pathway for mitotic chromosome formation**. *Science* 2018, **359**:eaao6135 http://dx.doi.org/10.1126/science.aao6135.
Time-resolved Hi-C through mitotic compaction reveals loss of compartmentalization and TADs during over the course of this process. Modeling reveals the structure of the mitotic chromosome as an array of nested loops emanating from a spiral scaffold. The loops get progressively larger through prophase. Knockouts of condensin I and II reveal their different role in mitotic compaction.

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

63. Mullenders J, Aranda-Orgilles B, Lhoumaud P, Keller M, Pae J, Wang K, Kayembe C, Rocha PP, Raviram R, Gong Y *et al.*: **Cohesin loss alters adult hematopoietic stem cell homeostasis, leading to myeloproliferative neoplasms**. *J Exp Med* 2015, **212**:1833-1850.

64. Viny AD, Ott CJ, Spitzer B, Rivas M, Meydan C, Papalexi E, Yelin D, Shank K, Reyes J, Chiu A *et al.*: **Dose-dependent role of the cohesin complex in normal and malignant hematopoiesis**. *J Exp Med* 2015, **212**:1819-1832.

65. Canzio D, Nwakeze CL, Horta A, Rajkumar SM, Coffey EL, Duffy EE, Duffié R, Monahan K, O'Keeffe S, Simon MD *et al.*: **Antisense lncRNA transcription mediates DNA demethylation to drive stochastic protocadherin α promoter choice**. *Cell* 2019, **177**:639-653.e15 http://dx.doi.org/10.1016/j.cell.2019.03.008.

66. Guo Y, Monahan K, Wu H, Gertz J, Varley KE, Li W, Myers RM, Maniatis T, Wu Q: **CTCF/cohesin-mediated DNA looping is required for protocadherin α promoter choice**. *Proc Natl Acad Sci U S A* 2012, **109**:21081-21086.

67. Rowley MJ, Jordan Rowley M, Nichols MH, Lyu X, Ando-Kuri M, Rivera ISM, Hermetz K, Wang P, Ruan Y, Corces VG: **Evolutionarily conserved principles predict 3D chromatin organization**. *Mol Cell* 2017, **67**:837-852.e7.

68. Haddad N, Jost D, Vaillant C: **Perspectives: using polymer modeling to understand the formation and function of nuclear compartments**. *Chromosome Res* 2017, **25**:35-50.

69. Di Pierro M, Zhang B, Aiden EL, Wolynes PG, Onuchic JN: **Transferable model for chromosome architecture**. *Proc Natl Acad Sci U S A* 2016, **113**:12168-12173.

70. Michieletto D, Chiang M, Colì D, Papantonis A, Orlandini E, Cook PR, Marenduzzo D: **Shaping epigenetic memory via genomic bookmarking**. *Nucleic Acids Res* 2018, **46**:83-93.

71. Jost D, Vaillant C: **Epigenomics in 3D: importance of long-range spreading and specific interactions in epigenomic maintenance**. *Nucleic Acids Res* 2018, **46**:2252-2264.

72. Leibler L: **Theory of microphase separation in block copolymers**. *Macromolecules* 1980, **13**:1602-1617.

73. Rubinstein M, Colby R: *Polymer Physics*. Oxford University Press; 2003.

74. Chandra T, Ewels PA, Schoenfelder S, Furlan-Magaril M, Wingett SW, Kirschner K, Thuret J-Y, Andrews S, Fraser P, Reik W: **Global reorganization of the nuclear landscape in senescent cells**. *Cell Rep* 2015, **10**:471-483.

75. Chiang M, Michieletto D, Brackley CA, Rattanavirotkul N, Mohammed H, Marenduzzo D, Chandra T: **Lamina and heterochromatin direct chromosome organisation in senescence and progeria**. *bioRxiv* 2018 http://dx.doi.org/10.1101/468561.

76. •• Falk M, Feodorova Y, Naumova N, Imakaev M, Lajoie BR, Leonhardt H, Joffe B, Dekker J, Fudenberg G, Solovei I *et al.*: **Heterochromatin drives organization of conventional and inverted nuclei**. *bioRxiv* 2018 http://dx.doi.org/10.1101/244038.
Using cells with natural and synthetically induced nuclear inversion, this study found that compartmentalization, as seen in microscopy and Hi-C, requires attraction between regions of heterochromatin. Modeling showed lamina–heterochromatin and euchromatin-euchromatin attractions to be dispensable for establishing compartments, with the attraction of lamina to heterochromatin being necessary for localization of the heterochromatin compartment to the nuclear periphery.

77. Solovei I, Kreysing M, Lanctôt C, Kösem S, Peichl L, Cremer T, Guck J, Joffe B: **Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution**. *Cell* 2009, **137**:356-368.

78. Luperchio TR, Sauria MEG, Hoskins VE, Xianrong W, DeBoy E, Gaillard M-C, Tsang P, Pekrun K, Ach RA, Yamada A *et al.*: **The repressive genome compartment is established early in the cell cycle before forming the lamina associated domains**. *bioRxiv* 2018 http://dx.doi.org/10.1101/481598.

79. Nuebler J, Fudenberg G, Imakaev M, Abdennur N, Mirny LA: **Chromatin organization by an interplay of loop extrusion and compartmental segregation**. *Proc Natl Acad Sci U S A* 2018, **115**:E6697-E6706.

80. Erceg J, Abed JA, Goloborodko A, Lajoie BR, Fudenberg G, Abdennur N, Imakaev M, McCole RB, Nguyen SC, Saylor W *et al.*: **The genome-wide, multi-layered architecture of chromosome pairing in early *Drosophila* embryos**. *bioRxiv* 2018 http://dx.doi.org/10.1101/443028.

81. Abed JA, Erceg J, Goloborodko A, Nguyen SC, McCole RB, Saylor W, Fudenberg G, Lajoie BR, Dekker J, Mirny LA *et al.*:

Highly structured homolog pairing reflects functional organization of the *Drosophila* genome. *bioRxiv* 2018 http://dx.doi.org/10.1101/443887.

82. Stanyte R, Nuebler J, Blaukopf C, Hoefler R, Stocsits R, Peters J-M, Gerlich DW: **Dynamics of sister chromatid resolution during cell cycle progression**. *J Cell Biol* 2018, **217**:1985-2004 http://dx.doi.org/10.1083/jcb.201801157.

83. Guacci V, Koshland D, Strunnikov A: **A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae***. *Cell* 1997, **91**:47-57.

84. Michaelis C, Ciosk R, Nasmyth K: **Cohesins: chromosomal proteins that prevent premature separation of sister chromatids**. *Cell* 1997, **91**:35-45.

85. Busslinger GA, Stocsits RR, van der Lelij P, Axelsson E, Tedeschi A, Galjart N, Peters J-M: **Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl**. *Nature* 2017, **544**:503-507.

86. Heinz S, Texari L, Hayes MGB, Urbanowski M, Chang MW, Givarkes N, Rialdi A, White KM, Albrecht RA, Pache L *et al.*: **Transcription elongation can affect genome 3D structure**. *Cell* 2018, **174**:1522-1536.e22.

87. • Bonev B, Mendelson Cohen N, Szabo Q, Fritsch L, Papadopoulos GL, Lubling Y, Xu X, Lv X, Hugnot J-P, Tanay A *et al.*: **Multiscale 3D genome rewiring during mouse neural development**. *Cell* 2017, **171**:557-572.e24.
This study uses ultra-high resolution Hi-C to compare mouse ES cells and derived neural cells, and characterizes patterns of contact frequency between functional sites on DNA (e.g. polycomb, TF sites, gene bodies) beyond the baseline set by compartmentalization.

88. Quinodoz SA, Ollikainen N, Tabak B, Palla A, Schmidt JM, Detmar E, Lai MM, Shishkin AA, Bhat P, Takei Y *et al.*: **Higher-order inter-chromosomal hubs shape 3D genome organization in the nucleus**. *Cell* 2018, **174**:744-757.e24.

89. Chen Y, Zhang Y, Wang Y, Zhang L, Brinkman EK, Adam SA, Goldman R, van Steensel B, Ma J, Belmont AS: **Mapping 3D genome organization relative to nuclear compartments using TSA-Seq as a cytological ruler**. *J Cell Biol* 2018, **217**:4025-4048.

90. Monahan K, Horta A, Lomvardas S: **LHX2- and LDB1-mediated trans interactions regulate olfactory receptor choice**. *Nature* 2019, **565**:448-453 http://dx.doi.org/10.1038/s41586-018-0845-0.

91. Kundu S, Ji F, Sunwoo H, Jain G, Lee JT, Sadreyev RI, Dekker J, Kingston RE: **Polycomb repressive complex 1 generates discrete compacted domains that change during differentiation**. *Mol Cell* 2018, **71**:191.

92. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA: **A phase separation model for transcriptional control**. *Cell* 2017, **169**:13-23.

93. Wang S, Su J-H, Beliveau BJ, Bintu B, Moffitt JR, Wu C-T, Zhuang X: **Spatial organization of chromatin domains and compartments in single chromosomes**. *Science* 2016, **353**:598-602.

94. Boettiger AN, Bintu B, Moffitt JR, Wang S, Beliveau BJ, Fudenberg G, Imakaev M, Mirny LA, Wu C-T, Zhuang X: **Super-resolution imaging reveals distinct chromatin folding for different epigenetic states**. *Nature* 2016, **529**:418-422.

95. Flyamer IM, Gassler J, Imakaev M, Brandão HB, Ulianov SV, Abdennur N, Razin SV, Mirny LA, Tachibana-Konwalski K: **Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition**. *Nature* 2017, **544**:110-114.

96. van Steensel B, Belmont AS: **Lamina-associated domains: links with chromosome architecture, heterochromatin, and gene repression**. *Cell* 2017, **169**:780-791.

97. Condensin II inactivation in interphase does not affect chromatin folding or gene expressionNezar Abdennur, Wibke Schwarzer, Aleksandra Pekowska, Indra Alon Shaltiel, Wolfgang Huber, Christian H Haering, Leonid Mirny, François SpitzbioRxiv 437459; doi: https://doi.org/10.1101/437459