

Review

The chromosome folding problem and how cells solve it

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SUMMARY

Every cell must solve the problem of how to fold its genome. We describe how the folded state of chromosomes is the result of the combined activity of multiple conserved mechanisms. Homotypic affinity-driven interactions lead to spatial partitioning of active and inactive loci. Molecular motors fold chromosomes through loop extrusion. Topological features such as supercoiling and entanglements contribute to chromosome folding and its dynamics, and tethering loci to sub-nuclear structures adds additional constraints. Dramatically diverse chromosome conformations observed throughout the cell cycle and across the tree of life can be explained through differential regulation and implementation of these basic mechanisms. We propose that the first functions of chromosome folding are to mediate genome replication, compaction, and segregation and that mechanisms of folding have subsequently been co-opted for other roles, including long-range gene regulation, in different conditions, cell types, and species.

In 1970, Ris and Kubai wrote the following¹: “the analysis of chromosome structure seeks to describe the spatial relationships of the various molecular components of chromosomes and to relate changes in these configurations to chromosome functions such as replication, transcription, and genetic recombination.” Around the same time Thomas wrote as follows²: “we do not know how chromosomes are organized, but there are some tantalizing clues, and we may be on the edge of finding out.”

Ris and Kubai described the challenges in the field of chromosome biology, which holds true to this day. Thomas was correct when he said that we were going to find out, although it took decades for the development of new methods, the contributions from different disciplines ranging from molecular to cell biology to evolutionary biology, and the sequencing of complete genomes. Parallel developments in physics continued changing our view of the nature of chromosomes, further pushing the envelope of Polymer Physics. Today, we know at least about some of the structures, molecules, and mechanisms driving chromosome folding. Yet how these processes and resulting chromosome structures relate to chromosome function continues to be a topic of intense study and debate. Still, there is the same sense of optimism that we may be on the edge of finding out the connections between folding and the working of the genome.

THE VIEW OF CHROMOSOMES 50 YEARS AGO

Among the first articles in *Cell* covering chromatin structure, chromosome folding, and nuclear organization were studies on fine-scale organization of eukaryotic chromatin fibers as “beads-on-a-string,”³ formation of histone tetramers,⁴ folded

prokaryotic nucleoids,⁵ and speculations on folding of DNA within the peculiar nuclei of dinoflagellates,⁶ eukaryotes with apparent liquid crystalline chromosomes that have become to the topic of renewed interest recently.^{7,8}

In the 1960s, it was known that chromosomes were each composed of a single long strand of DNA, and the question of how that DNA was spatially arranged to fulfill its role as genetic carrier was only just begun to be asked, and few answers were available. At first glance, it appeared that there were few commonalities between the structure of chromosomes from organisms from different kingdoms (e.g., eukaryotes vs. prokaryotes), between chromosomes from different tissues within a species (e.g., *Drosophila* polytene chromosomes in salivary glands vs. more conventional eukaryotic chromatin in other tissues), or between chromosomes observed at different stages of the cell cycle for any one cell type (the classical X-shaped compacted mitotic chromosomes readily visible in the light microscope vs. interphase chromosomes that essentially disappeared from view). Chromosomes could be linear, circular, regularly packed in proteinaceous capsids in phages, lightly packed in nucleoids in bacteria, wrapped around nucleosomes in (most) eukaryotes, supercoiled or not, and sometimes arranged in loops.

As for many other fields, the study of chromosome folding awaited the development of new technologies and assays that would ultimately allow the visualization of entire genomes at near base-paired resolution in 3D within single cells. These developments were complemented by the joining together of scientists from a range of disciplines, including cell biology, molecular biology, and structural biology, on the one hand, and physics, computational biology, and bioinformatics on the other.



This interdisciplinary effort has over the last few decades started to identify common principles of chromosome folding across the tree of life.

THE NEED TO FOLD CHROMOSOMES

In all organisms, the lengths of their genomes are large compared with the dimensions of the cell or nucleus. Stretched out, the genome of *E. coli* is ~1.7 mm long, compared with a cell diameter of 2 μm . The length of the human genome in all chromosomes is ~2 m, and the cell nucleus is only ~5–10 μm in diameter. However, given that DNA fibers are so thin (2 nm of DNA or 11 nm of the chromatin fiber), the volume of the genome fits very comfortably in the cell or nucleus. So, is there really a need for cells to actively organize and fold chromosomes?

There are strong arguments for why cells need to actively fold their chromosomes. First, assuming that chromatin behaves as an “unconstrained” polymer (i.e., an ideal chain, resembling a random walk) and that it has a persistence length of a ~70 nm containing ~3–5 kb,^{9,10} the diameter of an unconstrained chromosome of the length of just 1 copy of human chromosome 1 would be about 16 μm ($R_g = \sqrt{250,000/3} \times 70/\sqrt{6} = 8 \mu\text{m}$; diameter of the coils is twice that: 16 μm), exceeding that of the whole nucleus. Clearly, extensive compaction is required to fit in all 46 chromosomes.

Second, we previously¹¹ outlined that the polymer state of chromosomes has important implications for which loci can physically interact (e.g., genes and their regulatory elements), the kinetics by which such interactions can form, and in what fraction of cells such interactions can occur. In the absence of any constraints or active processes folding chromatin, short-range interactions between genomic loci, e.g., separated by up to tens of kilobases, will be frequent enough to occur in most cells in a reasonable amount of time (e.g., the duration of the cell cycle). However, longer-range interactions will be rare (~6% of the time for 0.5-Mb separation¹²) and will not be formed in most cells, even at very large timescales. Finally, in the absence of active and controlled folding processes, it is hard to imagine how specificity in interactions can be obtained (below).

Third, in the absence of active management of chromosome organization, any genomic process would be compromised. For instance, replicating long DNA molecules leads to pairs of sister DNAs that are topologically intertwined, creating a significant challenge to the cell, as was realized by Delbrück already in 1956.¹³ To segregate these sister molecules to daughter cells, cells need to compact each so that they are short and rigid to facilitate segregation, while simultaneously also topologically unlinking them. This process is fundamental to life and is intuitively the most obvious case for the need for processes to actively fold chromosomes.

THE CHROMOSOME FOLDING PROBLEM

The (cell-type-dependent) folding of chromosomes is related to the (cell-type-dependent) linear epigenome patterning along the genome: the presence, location, and activity of *cis*-regulatory elements such as enhancers, insulators and promoters, and (in eukaryotes) the presence of regions of specific combinations

of histone modifications that define different chromatin states including euchromatin and heterochromatin. Like the protein folding problem, defined as the question of how the primary amino acid sequence of a protein dictates its 3D folding, the chromosome folding problem can be defined as the question of how the linear epigenome is related to the spatial arrangement and folding of chromosomes in the cell.

However, and this is different from protein folding (see Mirny¹⁴ for review), chromosome folding is not only driven by affinities and interactions between genomic elements, it also involves biological activities that directly fold and refold chromosomes, and these molecular processes can in some cases fold chromosomes in ways largely unrelated to the linear epigenome, e.g., in mitosis.^{15–20} Chromosome conformation is also highly variable between individual cells, a result of the very large length of chromosomes, combined with their stochastic dynamics of self-assembly,^{21–23} and the action of highly dynamic folding processes such as loop extrusion, which rearrange chromosomes at the scale of hundreds of kilobases over tens of minutes. Furthermore, chromosomes can rapidly, in mere minutes, change their folding state, e.g., during entry and exit of mitosis.^{16,24–27}

We propose a more expansive definition of the chromosome folding problem: the question of how biophysical forces and molecular mechanisms, through the action of specific folding machineries, act on the linear epigenome to dynamically fold and refold chromosomes at different lengths and timescales, e.g., during the cell cycle, development, and other biological transitions.

Chromosome folding appears to differ in dramatic ways between species from different kingdoms (e.g., prokaryotes vs. eukaryotes) and as cells progress through the cell cycle. Such observations suggest that many different solutions to the chromosome folding problem may exist and that species- and condition-specific folding mechanisms must have evolved.

One of the most exciting discoveries of the last two decades has been that only a small number of universal biophysical and molecular processes drive chromosome folding. These major folding mechanisms are deeply conserved across the tree of life but are directed, regulated, and deployed in different ways to produce different folded states of chromosomes to accommodate the many different functions of genomes.

BREAKTHROUGHS IN DETERMINING CHROMOSOME FOLDING

Chromosomes were first described using microscopic methods. Given that initially only large mitotic and meiotic chromosomes in plants and amphibians could be individually observed by microscopic means, initial studies focused on these chromosomes. First concepts of chromosome folding in mitosis developed the notion of the “folded fiber,” ranging from irregular fibers²⁸ to radial loop structures^{29,30} to hierarchical models.^{19,31–34} Initial physical models of interphase chromosomes started to arise when fluorescence *in situ* hybridization (FISH) imaging established how the spatial distance between loci increases with the genomic separation between the probes. The first quantitative models considered an interphase chromosome as a random-walk polymer³⁵ or a confined or tethered polymer.³⁶ Alternative

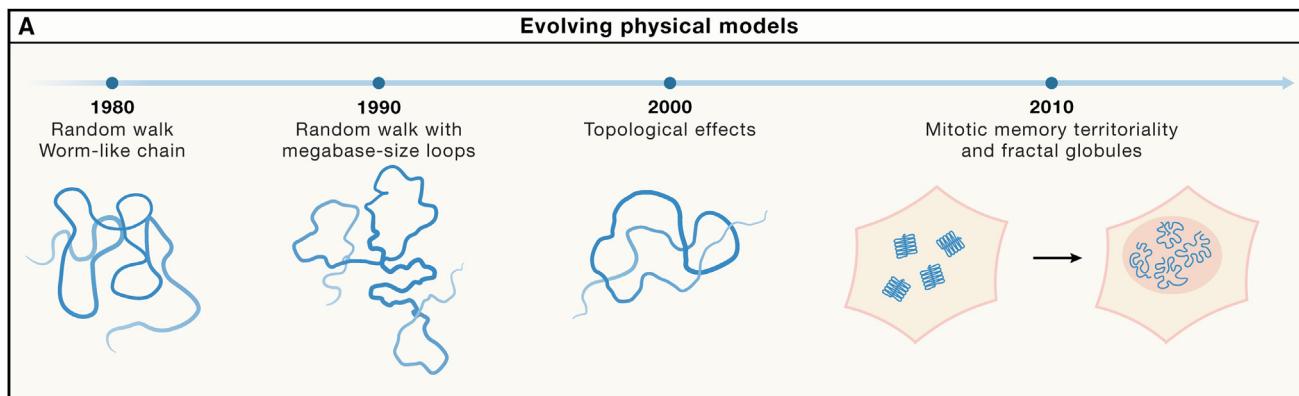


Figure 1. Evolving physical models over the last decades

Over the last 50 years, Polymer Physics has put forth increasingly refined models of chromosome conformation. See text for details.

models were proposed where chromosomes were folded into megabase-size loops along otherwise random-walk polymers^{37,38} (Figure 1).

Over the last two decades, four important developments have greatly enhanced and transformed the study of chromatin, chromosomes, and entire genomes.

The first of which was the ability to determine the sequence of complete genomes for many species. While initial genome sequencing efforts in the 1990s focused on smaller genomes of bacteria and some model organisms (e.g., budding yeast *S. cerevisiae*,³⁹ the nematode *C. elegans*,⁴⁰ and the fruit fly *D. melanogaster*⁴¹) and large-scale international efforts were required to sequence the mouse and human genomes,^{42–44} further increases in throughput and lower costs now enable the sequencing of any number of species and individuals. Now (near) full-length genome sequences are available for thousands of species, ranging from bacteria, to protozoa, archaea, fungi, plants, birds, mammals, etc. (e.g., Christmas et al.,⁴⁵ Stiller et al.,⁴⁶ and Wang et al.⁴⁷). Notably, Hi-C can also be used to assemble the linear genome (first shown by Kaplan and Dekker⁴⁸ and Burton and co-workers⁴⁹ in 2013) and is now routinely used to assemble genomes of new species (e.g., Dudchenko et al.⁵⁰ and Hoencamp et al.,⁵¹ and see Obinu et al.⁵² for an evaluation of these approaches).

Second, the development of genomic methods to probe the folding of chromosomes now allows for mapping chromosome structure directly to genome sequence. Some of these methods are based on chromosome conformation capture (3C,⁵³ 4C,⁵⁴ 5C,⁵⁵ Hi-C,⁵⁶ Micro-C,⁵⁷ DNase-Hi-C,⁵⁸ Chia-PET,⁵⁹ HiChIP,⁶⁰ Plac-seq,⁶¹ etc.), while other methods rely on mapping DNA sequences near sub-nuclear structures such as DamID,⁶² TSA-seq,⁶³ or the identification of loci co-located in clusters or specific sections of the nucleus (GAM⁶⁴, SPRITE,⁶⁵ etc.). In recent years, the resolution of these methods has increased so that 3D maps of genomes can be acquired at sub-kilobase resolution, as well as in single cells (e.g., Nagano et al.,⁶⁶ Nagano et al.,⁶⁷ Ramani et al.,⁶⁸ Li et al.,⁶⁹ Kind et al.,⁷⁰ and Tan et al.⁷¹).

Third, the development of imaging methods that can analyze the spatial locations of thousands of loci, so that the 3D struc-

ture of entire chromosomes, or even genomes, can be traced in single cells. These include large-scale locus tracing^{72,73}, OligoStorm and OligoDNA Paint,^{74,75} and ORCA.⁷⁶ Those paralleled developments of live-cell “tracking” of chromosomal dynamics that sheds light on underlying folding processes.^{12,77}

The fourth aspect includes developments in the understanding of chromosome folding from the polymer physics point of view: from the random-walk³⁵ and worm-like chain models in the early 1990s³⁶ to early models of chains with loops,^{37,38} to appreciation of topological effects,^{78–80} to more recent studies of active polymers⁸¹ or polymer driven by motors (loop extrusion)^{82,83} and folded onto loops,⁸⁴ and to models of polymer dynamics and response to external forces.^{85,86}

The development and application of genomic and imaging-based methods to determine the structure of chromosomes have been extensively reviewed elsewhere,^{87–91} and we refer the reader to those reviews and the primary literature cited therein. Here, we focus on current views of what the chromosome structure is, under different conditions and in different species; the mechanisms by which this structure is formed; and how chromosome structure and function are related.

FOUR MECHANISMS FOR FOLDING CHROMOSOMES

Studies in many species have shown that they share key mechanisms by which they fold chromosomes. Here, we describe these mechanisms.

Compartmentalization

One of the first features described for the spatial organization of chromatin inside eukaryotic interphase nuclei is the spatial segregation of inactive heterochromatic chromatin from active euchromatin (Figure 2), as first described by Emil Heitz.⁹² Classic microscopy studies showed dense, compacted chromatin clustered near the nuclear periphery, while decondensed, open chromatin was located more centrally. Later studies showed that more gene-dense chromosomes tend to locate centrally, while gene-poor chromosomes are more peripheral in the

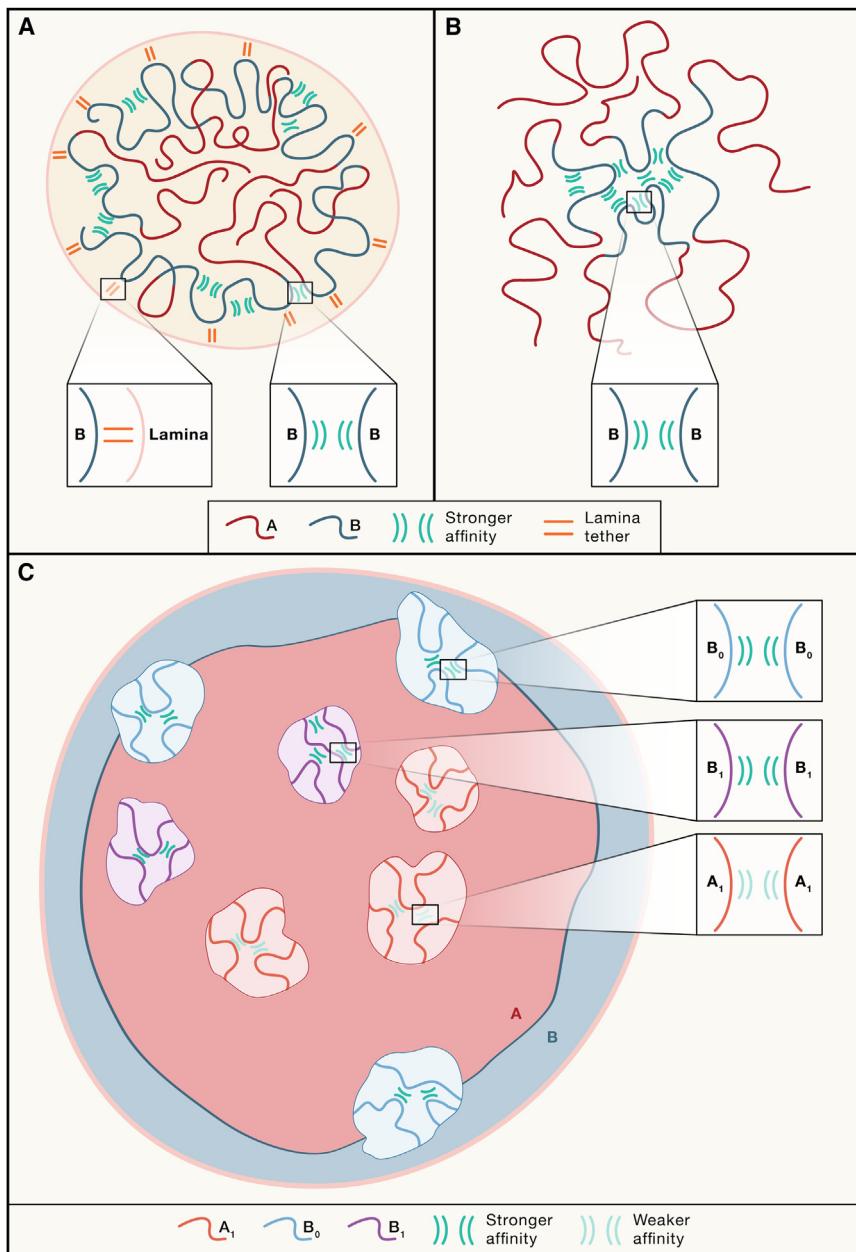


Figure 2. Two processes for nuclear organization: Compartmentalization through homotypic affinities and tethering to the nuclear periphery

(A) Eukaryotic chromosomes are composed of alternating A and B compartments. In conventional nuclear organization, strong B-B affinities lead to spatial separation of A and B compartments. A-A affinities are much weaker and contribute to a lesser extent. In addition, some B compartments are tethered to the nuclear periphery, resulting in enrichment of heterochromatin at the nuclear periphery, leaving euchromatin located centrally.

(B) In the absence of tethering of B compartment domains to the nuclear periphery, A/B compartmentalization occurs normally, but the strong B-B affinities result in clustering of all B compartments in the center of the nucleus, with A compartments located at the periphery (inverted nucleus).

(C) A more complex picture when more than two compartment types are present. A and B compartments can be split in different sub-compartments that can also display significant preferential homotypic affinities, leading to their spatial segregation.

nucleus.^{93–95} Studies on the timing of DNA replication also showed spatial segregation of early and late replicating chromatin, correlating with euchromatin and heterochromatin.⁹⁶

Genomic assays such as DamID directly mapped sequences near the nuclear lamina, again identifying regions poor in genes and mostly transcriptionally silent.^{97,98} The very first 4C and Hi-C datasets showed spatially segregated euchromatic and heterochromatic domains at genome-wide scale.^{54,56} In Hi-C, enriched interactions are readily detected between loci of similar chromatin and activity state: active and open chromatin interacts with other active and open loci, along the same chromosome (*cis*) and between chromosomes (*trans*). Similarly, inactive loci

interact with one another. This phenomenon is referred to as compartmentalization.

Initial lower-resolution Hi-C studies (megabase scale) showed the presence of just two types of chromatins that self-interact, and these two types (A and B compartments) had all the hallmarks of euchromatin and heterochromatin, respectively.⁵⁶ Subsequent higher-resolution Hi-C maps showed that each of these two major types of compartments can be split in so-called sub-compartments that differ in their precise chromatin composition, e.g., histone modification patterns, and can be as small as several kilobases.⁹⁹ Each of these sub-compartments displays characteristic patterns of long-range interactions with other loci, but most display a preference to interact with other loci of the same sub-compartment type (Figure 2A). A major recent

insight is that the number of types of sub-compartments is larger than previously anticipated (Figure 2C) and that these are not necessarily universally present, i.e., in a given cell type not all sub-compartment types may be observed.^{100,101}

Compartmentalization is thought to be driven by homotypic affinities between loci^{102–106} (Figure 2). The molecular nature of the factors that mediate these affinities are not known in detail. It is intriguing that compartmentalization has mostly been detected in eukaryotes that have nucleosomes and not in eukaryotes without nucleosomal DNA, e.g., dinoflagellates^{7,8} and in some archaea.¹⁰⁷ Although other explanations can be proposed, it may indicate a key role for histones in this process.

Compartmentalization is correlated with the presence of histone modifications: each sub-compartment has a characteristic combination of histone modifications.^{100,101,108} *In vitro*, short chromatin fibers carrying histone H3 lysine 9 trimethylation (H3K9me3) can form condensates, indicating that the modified histones themselves can play a role in the clustering of heterochromatin.¹⁰⁹ Furthermore, factors that recognize patterns of histone modification can act as bridging factors and, in that way, connect distal loci to stabilize compartmentalization. For instance, HP1 proteins can bind H3K9me3 and can bind multiple histone tails simultaneously. Such bridging factors can also phase separate themselves, leading to aggregation of such proteins together with multiple loci.^{110–114} While HP1 proteins can contribute to compartmentalization in that manner, loss of HP1 has surprisingly little effect on compartment formation,¹¹⁵ pointing to roles for other yet to be identified factors. Other examples are polycomb complexes that mediate deposition of histone H3 lysine 27 trimethylation (H3K27me3) modifications and then associate with chromatin carrying this mark, stabilizing long-range interactions in *cis* and in *trans* between loci silenced by these factors (for reviews of the extensive literature on this topic, see Akilli et al.¹¹⁶ and Schuetzengruber¹¹⁷). While many polycomb-bound loci reside in the larger A compartment and can be located in nuclei centers,¹¹⁸ they tend to engage in prominent long range with other polycomb-bound sites, e.g., in *Drosophila*¹¹⁹ and vertebrates, especially in embryonic stem cells.^{120,121}

Compartmentalization constitutes a phenomenon of microphase separation, i.e., a phenomenon where a polymer (chromosome) made of blocks of A and B monomers (or more types) forms spatially separated domains whose sizes depend on the sizes of the blocks in the sequence. Such a process can be driven by attractions between homotypic elements (A-A and/or B-B). Phase separation has also been seen in *in vitro* chromatin reconstruction experiments.¹⁰⁹ Initial studies demonstrated that the characteristic pattern of A/B compartmentalization seen in Hi-C data can be reproduced by models with homotypic affinities,^{106,122} yet the relative contributions of these affinities for A and B compartments remained unknown. A solution came from a rare biological system of the “inverted” nucleus in the rod photoreceptors.¹²³ Natural loss of attachment of heterochromatin to the nuclear periphery in such nuclei resulted in the repositioning of the heterochromatin to the center with the euchromatin taking peripheral locations. Both microscopy and Hi-C¹⁰⁴ confirmed that despite their inversion such nuclei are perfectly compartmentalized, demonstrating that compartmentalization is driven by interactions within chromatin rather than anchoring of heterochromatic loci to the lamina. Polymer models further demonstrated that homotypic affinities of heterochromatic regions drive compartmentalization, with affinities between euchromatic regions being much weaker (Figure 2B).

These predictions from models were confirmed with direct observation of dissociation kinetics of chromatin interactions by liquid chromatin Hi-C.¹²⁴ In liquid chromatin Hi-C, chromatin is fragmented *in situ*, leading to progressive dissociation of chromatin interactions over time, which can be measured using Hi-C. It was found that chromosomes remain compartmentalized even when chromatin is fragmented to an average size of 10–20 kb. When chromatin is fragmented to a size of less than 6 kb, inter-

acting chromatin segments dissociate within tens of minutes. Importantly, the kinetics of dissociation were related to the chromatin state, with heterochromatic loci dissociating slower than euchromatic loci, pointing to higher affinities between heterochromatic loci. Together with observations obtained with very deeply sequenced Hi-C datasets,⁹⁹ these data show that sub-compartments can be as small as several kilobases. Heterochromatic interactions between H3K9me3-marked loci were found to be more stable and thus contribute most strongly to compartmentalization, as predicted by modeling.¹⁰⁴

A mechanism of compartmentalization driven by largely relatively stable interactions between H3K9me3-marked heterochromatic loci does not rule out affinities between other regions, e.g., euchromatic loci (that among other factors can be direct or mediated by nuclear speckles) (Figure 2C). In fact, enrichment of contacts between histone H3 lysine 27 acetylation (H3K27ac) regions is evident from Micro-C^{125,126} by averaging over thousands of regions in Hi-C,¹²⁷ but it is most distinctly observed with region-capture Micro-C.^{128,129} While indicating that euchromatin regions also have some homotypic affinities, the need for averaging or for exceedingly deep sequencing suggest that such contacts are rare, consistent with microscopy.¹²⁷ Although they can help to stabilize otherwise transient interactions between regulatory elements and promoters at sub-megabase separations, the rarity of such contacts at larger genomic distances suggests few functional roles that they can play. Interactions between active loci can also be driven by bridging factors. For instance, Brd2/3/4 proteins can drive clustering of chromatin marked with H3K27ac both *in vivo* and *in vitro*.¹⁰⁹ A particularly interesting case is an oncogenic BRD4-NUT protein fusion that can lead to the spreading of H3K27ac through large regions, which in turn results in spatial clustering of such hyperacetylated “megadomains.”¹³⁰ This may be relevant for normal cells as well, where even relatively small H3K27ac-enriched loci such as enhancers and promoters can form small microcompartment domains.^{128,131,132}

An important aspect of compartmentalization as an affinity-driven process is that such clustering and spatial segregation will occur both in *cis* and in *trans*. This makes this process fundamentally different from other mechanisms of chromosome folding such as loop extrusion that acts strictly in *cis* (see below). Furthermore, compartmentalization is driven by local interactions leading to stochastic assemblies at the scale of whole chromosomes or genomes. In other words, in each cell, a different configuration is obtained, but in each cell, active loci preferentially cluster with other active loci, but which specific sets of loci cluster together can differ. The process results in stochastic co-localization of loci of similar chromatin state but has otherwise limited specificity in terms of which specific DNA sequences interact in any given cell. This aspect is important for understanding potential functional roles for compartmentalization.

Although compartmentalization is mostly observed in eukaryotes, the biophysical process that underlies this phenomenon, affinity-driven clustering loci, likely also occurs in prokaryotes (below).

Loops, extrusion, and their control

A first description of chromosome loops in mitotic chromosomes appeared in this journal in 1977,¹³³ leading to the radial loop

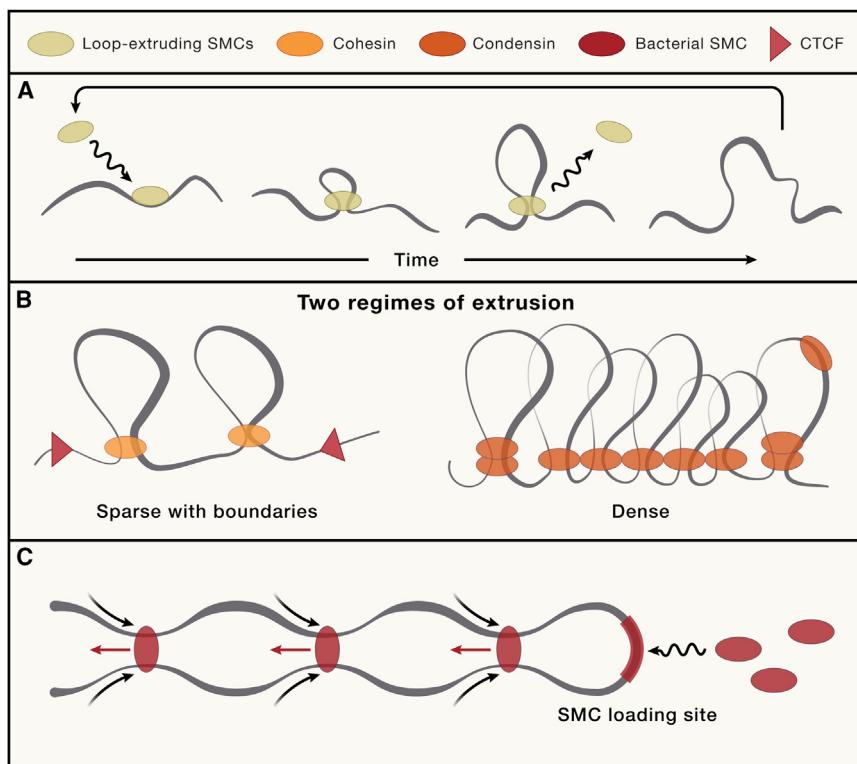


Figure 3. Two regimes of loop extrusion produce different conformations, consistent with interphase and mitosis

(A) Activity of a loop extruder: the complex loads and extrudes some amount of time after which it may dissociate or is actively unloaded.

(B) Left: During interphase (in vertebrates), cohesin is the main loop extrusion complex. It has a short residence time, generating a low density of transient loops, and the chromosomes appear diffuse in shape. Cohesin can be blocked by CTCF-bound sites, generating enrichment of positioned loops at these elements. Right: During mitosis, condensins are the main loop extrusion complexes. Condensin II has a long residence time, generating stable arrays of consecutive loops that lead to compaction into the rod-shaped mitotic chromosomes. Condensin is not blocked by CTCF, and the loop array is not positioned at reproducible loci in the cell population.

(C) In bacteria, repeated loading of loop-extruding complexes at defined loading sites can lead to juxtaposition of the chromosome arms, sequences on either side of the loading site.

model of mitotic chromosomes.^{30,134} Proposals about the presence of loops in interphase chromosomes have started to appear at about the same time, based on sedimentation data,^{135,136} and later as physical models fit to microscopy data.^{37,38,137}

What is now known as the process of loop extrusion (Figure 3) has a rich history. The ideas of enzyme-mediated loop growth started to emerge in the literature as many unrelated hypothetical mechanisms underlying VDJ recombination,¹³⁸ compaction of interphase chromosomes,¹³⁹ enhancer-promoter interactions,^{139,140} supercoiling,¹⁴¹ and mitotic compaction and segregation.¹⁴² Many works attributed these processes to structural maintenance of chromosomes (SMCs) complexes,^{143–145} with indications that SMCs can function as chromatin compacting motors.¹⁴⁶ In the context of mitotic compaction, loop extrusion was first mathematically modeled.¹⁴⁷ However, because of the lack of polymer models to make concrete predictions following from the activity of these mechanisms, and experimental data to test and validate the models, these proposals remained largely hypothetical.

Emergence of chromosome conformation capture data provided rich grounds for developing and testing mechanisms of chromosome folding by loop extrusion. One prediction of the theory¹⁴⁷ was that achieving mitotic compaction would lead to the formation of a loop array where non-overlapping loops follow each other (if extruders cannot bypass each other). Indeed, 5C and Hi-C data for mitotic cells were found to be consistent with such organization of loops.¹⁵ Polymer simulations further indicated that loop extrusion can compact and segregate polymers

of chromatids leading to morphologies of simulated chromosomes resembling those of early prophase chromosomes.¹⁴⁸ Loop extrusion during interphase^{149,150} was suggested as a mechanism underlying the then recently discovered topologically

associating domains (TADs)^{151,152} and associated features such as stripes and dots observed in Hi-C interaction maps.¹⁰⁰ These features emerge naturally in polymer models of loop extrusion when extrusion is occluded by boundaries. Modeling studies also suggested that loop-extruding motors are SMC complexes: cohesins in interphase and condensins during mitosis, while extrusion barriers are DNA-bound CCCTC-binding factor (CTCF) proteins. The loop extrusion hypothesis gained broad support by CTCF and cohesin depletion^{153,154} and modulation experiments, all generating model-predicted outcomes.¹⁵⁵ Direct visualization and characterization of loop extrusion by SMCs in single-molecule experiments (see Davidson and Peters¹⁵⁶ and Hoencamp and Rowland¹⁵⁷ for reviews) demonstrated that these complexes are indeed loop extrusion motors, as anticipated.^{142,147,149} The demonstration that the same mechanism in different regimes¹⁵⁸ can lead to either interphase organization or to mitotic compaction (Figure 3B) suggests that loop extrusion by SMCs can be a universal mechanism organizing chromosomes.

SMCs are ring-shaped and flexible protein complexes and include cohesin, condensin, SMC5/6, their bacterial counterparts, and possibly other complexes involved in DNA repair.¹⁵⁷ While condensins were known to be essential for mitotic chromosome compaction,^{159–161} their mode of action was long unknown. Cohesin has been characterized as a complex that keeps two sister chromatids together.¹⁴² Predicted loop extrusion activity of SMCs^{147,149} was initially a surprise, but single-molecule experiments have definitively demonstrated that SMC complexes can extrude loops in an ATP-dependent

manner.^{162–166} In such *in vitro* experiments with naked DNA as template, as well as in live cells on endogenous chromatin,^{12,24} loop extrusion is fast: ~1–3 kb/s. Different SMCs were found to be either one-sided or two-sided loop extruders,^{162–165} with two-sided extrusion possibly resulting from rapid switching of one-sided extrusion activity.¹⁶⁷ These complexes have relatively low stall forces^{162,164} (i.e., forces of 0.1–1 pN suffice to stop extrusion), and some complexes such as cohesin are blocked by obstacles such as RNA polymerases,¹⁶⁸ CTCF, and minichromosome maintenance (MCM) proteins (the latter two through a specific protein-protein interaction^{169,170}). On the other hand, condensins display the surprising ability to bypass each other¹⁷¹ or obstacles much bigger than their size.¹⁷² Yet the molecular mechanism of loop extrusion and force generation remains enigmatic and an area of active research.^{173,174}

These developments paralleled studies of SMCs and their activity in bacteria. 5C and Hi-C in *B. subtilis*¹⁷⁵ and *C. crescentus*^{176,177} revealed folding of the chromosomes “in half” with two juxtaposed arms, in an SMC-dependent manner. Further studies not only indicated loading of some SMCs at specific sites (origin-proximal in many species, e.g., at ParS sites near the origin in *B. subtilis*¹⁷⁸) but also directly visualized how such loading resulted in a progressive juxtaposition of the arms (Figure 3C). Strikingly, ahead of studies in eukaryotes, time-resolved Hi-C in bacteria allowed for measuring the speed of loop extrusion in living cells at ~1 kb/s.¹⁷⁹

Our understanding of the loop extrusion mechanism has significantly progressed in recent years. Practically every assumption and prediction of the original loop extrusion model has been challenged and mostly confirmed. As anticipated, cohesin depletion leads to an increase in distances between all loci as seen by chromatin tracing,⁷³ and ~50% of chromatin is located in extruded loops at any moment.¹² Many processes and complexes on crowded DNA function as barriers to loop extrusion, including the process of transcription,¹⁶⁸ elements of the replicative machinery,¹⁸⁰ even in G1. CTCF remains the strongest known barrier that relies on a specific peptide that can halt extrusion.^{100,153,169} Single CTCF sites, however, are permeable¹⁸¹ and loops that bridge two CTCF sites are rare and transient.^{77,12} Broadly, these findings indicate that patterns of contacts formed by loop extrusion are transient, suggesting that it is the process of extrusion rather than specific patterns that can play functional roles.^{182,183}

Several “rules of engagement” for SMCs, which determine how encounters between different complexes along the chromosome are resolved, are being discovered^{24,184} (Figure 4): they can block each other (likely cohesins¹⁸⁴), one triggering unloading of the other (e.g., condensins triggering unloading of extruding cohesins²⁴), or they can bypass each other and thus form more complex overlapping loops (as seen in single-molecule experiments^{171,172} and in bacteria¹⁸⁵). Interactions of loop-extruding SMCs with SMCs holding sisters chromatids (“cohesive cohesins”) can vary as well, with yeast cohesins stopping at sites of sister cohesion,¹⁸⁴ while animal condensins bypass such sites in mitosis.²⁴

Many of these mechanisms can be controlled by genomic elements and epigenetic context.^{183,186} Examples known so far include methylation-dependent CTCF binding, targeted loading

(at ParS sites in bacteria,¹⁸⁷ and likely loading at active enhancers^{183,186,188} but not at promoters in animals), domains of localized extrusion activity (suggested in the silkworm¹⁸⁹), and sites of SMC unloading (e.g., 3' ends of active genes in human¹⁸³ and mouse¹⁹⁰). The role of epigenetic context in regulating loop extrusion is less well understood. While extrusion is active in both euchromatin and heterochromatin compartments,¹⁰¹ heterochromatin is refractory to CTCF binding and hence devoid of boundaries.

Learning rules in one organism and extrapolating these to others, we anticipate that (1) the speed of extrusion, loading, and unloading can be controlled epigenetically in animals; (2) replication forks and likely other genomic processes can halt/pause extrusion, thus breaking or establishing extrusion-mediated interactions; and (3) extrusion activity can be non-uniform along the genome. Broadly, changes in extrusion-mediated patterns through differentiation and development suggest that epigenetic marks can control extrusion and barriers. Extrusion may in turn play a role in the localization and spreading of epigenetic marks, as suggested by CTCF-demarcated domains of gamma-H2AX spreading upon double-stranded break (DSB) repair.^{191,192}

Associations with landmarks of the nucleus

In eukaryotes, loci can become tethered to the nuclear periphery and the nucleolus as well as other structures such as nuclear speckles that are enriched in RNA processing and splicing factors. In prokaryotes specific loci can be found tethered to the cell wall, e.g., the ParS sites in *C. crescentus* are tethered to the wall at one pole of the elongated cell.

The molecular mechanisms of tethering are becoming clearer only for a few of such associations. In vertebrates, tethering of heterochromatic domains to the nuclear periphery has been studied extensively. Large domains, referred to as lamin-associated domains or LADs, are found associated with the nuclear periphery.^{97,98} These domains are enriched in particular histone modifications such as H3K9me3 and H3K9me2 and are typically transcriptionally silent and compacted. Lamins may not be exclusively involved,^{193,194} and other factors such as the lamin B receptor have been found to play roles.¹⁹⁵ However much less is known about the factors that determine clustering of loci around nucleoli or speckles, but a role for CTCF has been proposed.¹⁹⁶

The functional relevance of tethering loci at sub-nuclear structures is largely unknown. While most cells have heterochromatic domains localized at the nuclear periphery, in specialized cell types such as rod cells, heterochromatin is not tethered and now is instead localized in the center of the nucleus.¹²³ This does not affect compartmentalization and does not appear to have dramatic effects on gene expression. It should also be noted that some active chromatin can be found localized at the nuclear periphery as well, especially around nuclear pores. The functional relevance of these associations is not established, but one possible role could be that this would facilitate rapid mRNA export (“gene gating”).¹⁹⁷

In other organisms or conditions, tethering of loci to the cell wall (bacteria¹⁹⁸) or the nuclear envelope is critical for chromosome segregation or chromosome pairing (e.g., meiosis I¹⁹⁹). Although tethering is a straightforward way to facilitate spatial positioning

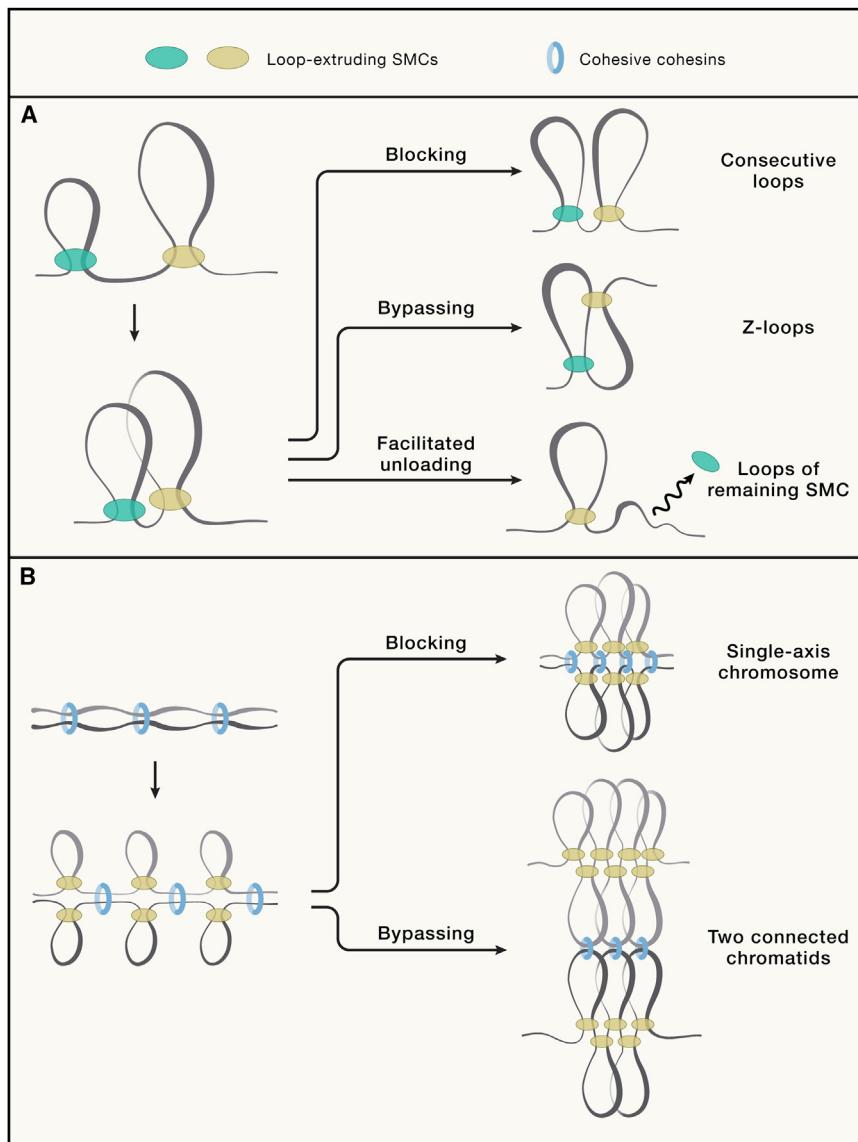


Figure 4. Rules of engagement for different SMCs result in different loop organization and structures of compacted chromosomes

(A) Three possible outcomes of an encounter between loop-extruding SMCs (green and yellow): they can block each other, leading to formation of consecutive loops; bypass each other forming so-called Z-loop¹⁷¹; or one can facilitate dissociation of another (other outcomes are also possible, e.g., one pushing the other back, etc.).

(B) Two possible outcomes of interactions between cohesive cohesins (blue rings) and loop-extruding SMCs (yellow). Top: when extruders are blocked by cohesive complexes, sister chromatids are predicted to be connected at the bases of the loops, forming a single axis (as in meiotic prophase I and early mitotic prophase). When extruders can bypass cohesive complexes, sister chromatids are predicted to be connected through the tips of their loops (as in mitotic prometaphase).

can lead to two phenomena: (1) exceedingly slow mixing between chromosomes after exit from mitosis, leading to the formation of chromosomal territories; and (2) slow equilibration within each chromosome, leading to the folding of the chain into a non-equilibrium and long-lived hierarchically organized and unknotted state known as the fractal (or “crumpled”) globule.⁷⁹

Polymer simulations and analysis of microscopy data for *Drosophila* suggested that polymers within each chromosome are folded into such a crumpled state,²⁰⁴ and they likely equilibrate exceedingly slowly. The first Hi-C data and polymer simulations provided compelling evidence that human interphase chromosomes are folded into the fractal globule state at the scale below ~10 Mb.⁵⁶ In this state, a chromosome resembles a “space-filling” curve, i.e., continuous regions of the chromosome form compact

of loci, much work is needed to explore the molecular players,¹⁹⁴ as well as roles of any *cis*-elements that participate in these events, and to explore the functional relevance.

Topological constraints

The role of topological effects in the way cells manage their exceedingly long chromosomes, disentangling strands, and compacting long chains have concerned biologists²⁰⁰ and physicists^{13,78} alike. Topoisomerase II was found to be essential for chromosome individualization²⁰¹ and was argued to be essential for fast mitotic compaction.⁷⁸ Effects of topological constraints and entanglements on polymer dynamics have been well known in physics²⁰² and hypothesized to impact the way the genome is folded.^{79,203}

Polymer theory suggested that the presence of topological constraints, i.e., when topoisomerase II activity is absent or very low,

spatial blobs, as observed by microscopy.²⁰⁵ The contact probability P between genomic loci decays with genomic distance (s) as $P(s) \sim s^{-\alpha}$ with $\alpha \approx 1-1.1$.²⁰⁶ This contrasts a polymer without topological constraints, i.e., strand passage can freely occur, which when compacted, resembles a random-walk configuration in a confinement, where short continuous regions are expanded rather than compacted, leading to a rapid decay of the contact probability with genomic distance ($\alpha \approx 1.5$) followed by a plateau. Interestingly, chromosomal arms of yeast *S. cerevisiae*, which are relatively short (up to 0.8 Mb) and do not have a compact mitotic state, show a random-walk folding ($\alpha \approx 1.5$).⁹ Multi-contact 3C data and polymer simulation for mammalian cells also demonstrate that each chromosome is largely unknotted.²⁰⁷ It remains to be seen whether such unknotted and locally compact fractal globule folding and chromosomal territories have any specific functional roles or simply

represent a memory of the unentangled telophase state²⁰⁸ preserved by topological constraints.

Recently, a more complex picture of how cells manage topological states of chromosomes started to emerge. Activity of topoisomerase II allows strand passage, turning the chain into a topologically unconstrained one, which can result in an increased or a decreased level of entanglement. Interestingly, loop extrusion can bias topoisomerase II activity toward unknotting an initially knotted chain.²⁰⁹ Extruded loops can also buffer topological interactions, making the chain less sensitive to topological constraints,⁸⁴ e.g., in interphase chromosomes. Self-entanglement of mitotic chromosomes have long been anticipated due to the critical role of topoisomerase II in mitotic compaction^{201,210,211} and as demonstrated in micromechanical experiments.²¹² A recent study showed that mitotic and interphase chromosomes have very different topological states, with mitotic chromosomes being highly self-entangled while interphase is relatively free of knots, and it suggested a pathway that allows cells to interconvert between them as cells exit mitosis.²⁰⁸ Yet to convert highly entangled mitotic chromosomes into an unentangled interphase state, cells require high activity of topoisomerase II during mitotic exit. To direct topoisomerase II activity toward unentanglement and then preserve this unentangled interphase state, a two-stage mitotic exit mechanism was proposed.²⁰⁸ At the first stage, decompaction while preserving mitotic loops biases topoisomerase II to disentangle the mitotic state, creating the unentangled compact state at telophase. During the second stage, chromosomes expand without much topoisomerase II activity, thus forming chromosomal territories and fractal globule states in G1.

Sister chromatids are initially topologically intertwined during and after S-phase. Such topological connections will maintain connections between sister chromatids even in the absence of cohesive cohesin complexes. For segregation, these intertwines need to be removed. Loop extrusion in the presence of topoisomerase II activity has been shown by modeling to drive compaction of each sister chromatid, while unlinking them.¹⁴⁸ In effect, extrusion pulls the sisters away from each other, which will drive the otherwise unbiased strand passage reaction by topoisomerase II toward decatenation.

THE PHYSICAL STATE OF CHROMOSOMES

While Hi-C provides crucial information about the global state of chromatin in the scaling of the contact probability with genomic distance $P(s) \sim s^{-\alpha}$,²⁰⁶ microscopy measures a complementary characteristic: spatial separation $R(s) \sim s^\nu$. Interphase animal chromosomes typically yield $\alpha \approx 1-1.2$ and $\nu \approx 0.25-0.3$,^{73,213} for $s \approx 0.5-10$ Mb, both consistent with the fractal (crumpled) globule folding, i.e., nearly space-filling organization where long continuous regions of the genome occupy continuous volumes in space. The fractal globule state is perturbed by extruded loops and thus is best visible when cohesin is depleted and in synchronized cells, leading to $P(s) \sim s^{-1.1}$ scaling from 10 kb to 10 Mb (e.g., Schwarzer et al.,²¹⁴ Hsieh et al.,¹²⁶ and Samejima et al.²⁴). In *S. cerevisiae*, Hi-C and microscopy yield $\alpha \approx 1.5$ and $\nu \approx 0.5$,²¹⁵⁻²¹⁷ both characteristic of random-walk chains, indicating

that yeast chromosomal arms—away from clustered centromeres and anchored telomeres—are largely unconstrained polymers.²¹⁵ In vertebrates, the fractal globule nature of folding is fully consistent with compartmentalization and loop extrusion.^{84,208} Yet the physical nature of this crumpled state, and thus the interphase vertebrate chromosomes, remains enigmatic due to difficulties in reconciling the fractal globule state with dynamics and force response.⁸⁶

Studies of chromosome dynamics provide a view complementary to that learned from Hi-C and microscopy. Moreover, timescales and frequencies of contacts measured by live-cell microscopy provide a foundation for understanding interactions between functional elements (see Grosse-Holz⁸⁶ and Tortora et al.²¹⁸ for reviews).

Early works in chromosome dynamics in vertebrates used histone-fused photoactivatable green fluorescent protein (GFP), allowing for tracking changes in patterns of global chromosome organization in the nucleus, and brought two key insights.²¹⁹ First, dynamics during interphase is rather slow with a displacement of ~ 1 μ m during an ~ 24 -h interphase. Second, a great deal of randomization of positions of individual loci occurs after a cell division.

Tracking of individual (or pairs of) loci in live cells allowed for quantifying mean-squared displacement (MSD) over an interval t , yielding $MSD \sim t^\mu$ with $\mu = 0.35-0.5$ in bacteria, yeast, fly, and mammalian cells.^{12,77,220-223} In *S. cerevisiae*, measured $\mu = 0.5$ ^{221,224} is in perfect agreement with Hi-C and microscopy and is characteristic of a motion of a locus of a flexible but otherwise unconstrained polymer (the so-called Rouse model). Surprisingly, most of the studies in animal cells also reported $\mu = 0.5$,^{12,77,225} which is hard to reconcile with $P(s)$ and $R(s)$ and broadly with the fractal globule that is expected to give $\mu = 0.2-0.4$ (see Tamm and Polovnikov²²⁶ for review). Such inconsistency between crumpled $R(s)$ and unconstrained MSD became most evident when both characteristics were measured using the same approach and in the same cells (see Grosse-Holz⁸⁶ and Brückner²²⁷ for reviews). Some studies in mammalian cells yielded $\mu = 0.2$ interpreted as a reflection of the properties of the nucleoplasm and suggesting a near gel state of the chromatin.²²² Live-cell measurements, however, and their analysis heavily rely on specifics of the experiment and correction for localization uncertainty (see Grosse-Holz⁸⁶ for review).

Live-cell tracking also estimates the time it takes for a chromosomal region to sample its conformations. For example, in mammalian cells it takes about 40 min for a chromosomal region of 0.5 Mb to sample its conformations,¹² only 5 min for two loci separated by 150 Mb to come sufficiently close ($\sim 100-200$ nm),⁷⁷ while a larger (~ 2 -Mb) region didn't equilibrate in 40 min.²²³ These times do not simply imply same times for functional molecular interactions between chromosomal loci. For example, CTCF sites separated by 0.5 Mb form a stable interaction only about once per day and require cohesin-mediated extrusion.¹² Broadly, it remains to be seen how dynamics and proximity translate into functional interactions, which can critically depend on the radii over which such interactions can be established and on the molecular context.

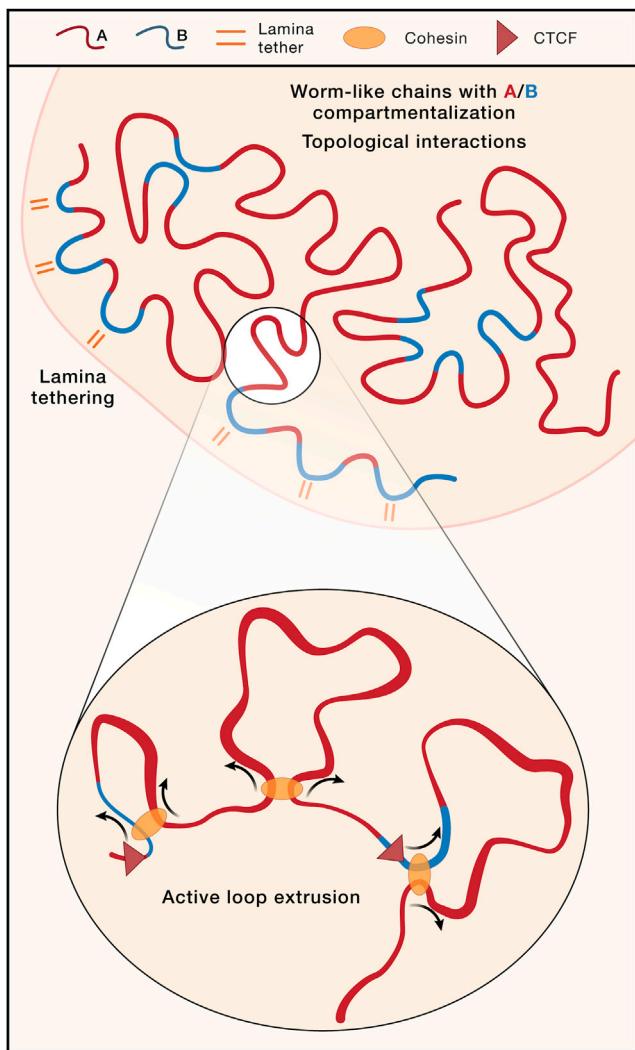


Figure 5. Current models of interphase chromosome organization through integrated activity of multiple mechanisms

Schematic depiction of interphase chromosome conformation in eukaryotes as the combined and integrated result of multiple folding mechanisms. The chromosome is a worm-like chain that phase separates in distinct compartments (A/B compartments or finer sub-compartments) driven by homotypic affinities. Tethering of domains to sub-nuclear structures, such as the nuclear lamina, the nucleolus, or nuclear bodies including speckles, leads to positioning of loci and chromosomes at specific nuclear locations. Topological constraints prevent mixing in interphase, but self-entanglements are formed in mitosis, facilitating full and fast compaction. At the scale of hundreds of kilobases, loop extrusion, guided by *cis*-elements that determine loading, unloading, and blocking (CTCF) of loop extruders, and with extensive interplay with other folding mechanisms, including compartmentalization, adds an additional layer of chromosome folding.

Studies of chromosome mechanics

Complementary to pictures obtained by microscopy and Hi-C are studies of chromosome mechanics. Loop formation and expansion and compaction/expansion of chromatin domains occur in the context of a crowded chromatin environment, leading to mechanical forces acting on chromosomes. Interplay between molecular processes that fold chromosomes and mechanical forces

have been proposed to guide mitotic and meiotic chromosome compaction and chromosome segregation.^{228–230}

Mechanical perturbation of the whole nucleus demonstrated that for small displacements the nuclear response is driven by elastic properties of the polymer of chromatin attached to the nuclear lamina, while for larger displacements it is the stretching of the lamina that determines the response.²³¹ Inducing elevated levels of histone methylation makes chromatin stiffer, while elevated histone acetylation makes it softer.²³² This suggests that in some cell types, chromatin may play a role in providing optimal mechanical properties of the nucleus.

Micromechanical studies of isolated human mitotic chromosomes²³⁰ have provided important insights by demonstrating (1) the extraordinary elasticity of chromosomes that are able to extend to more than five times their length²³³; (2) the role of histone methylation in rigidifying chromatin, consistent with self-affinity of such heterochromatin regions²³⁴; (3) the role of HP1alpha in mediating some of these interactions¹¹¹; and (4) the suggested key roles of condensins and topological entanglement in providing mechanical stability of mitotic chromosomes.²¹² Broadly, these studies suggested that significant crosslinking turns a mitotic chromosome into a gel.²³⁰ However, the nature of these crosslinks—topological vs. SMC vs. non-SMC based—are yet to be understood.

A recent study of interphase chromosomes was able to perform a pull-release mechanical perturbation in live human cells.⁸⁵ These experiments showed that chromosomes responded as almost unconstrained (Rouse) polymers, consistent with their dynamics (see above). Surprisingly, chromosomal loci could travel micrometers across the nucleus in mere minutes. Models of a free polymer subject to weak affinities to the surrounding media can reproduce this behavior of chromatin, arguing that interphase chromosomes, unlike mitotic ones, are not gel-like or crosslinked. In summary, developing a physical model of interphase chromosomes that can unify Hi-C, microscopy, live-cell dynamics, and mechanics is an important challenge.⁸⁶

FOLDING CHROMOSOMES THROUGH THE COMBINED ACTION OF DIFFERENT FOLDING MECHANISMS

The final folding state of a chromosome, or whole genome, is determined by the combined action of the several folding mechanisms described above^{22,235,236} (Figure 5). In addition to physical linkage, every locus is subject to the forces imposed by these mechanisms that combined determine its position with respect to other loci, its local dynamics, and its association with sub-nuclear structures such as the nuclear periphery or nuclear bodies including speckles and nucleoli. In addition, there is interplay between folding mechanisms, e.g., between loop extrusion and compartmentalization so that chromosome folding is not just the additive effect of each process in isolation. We discuss the folding of vertebrate interphase genome folding as an example given that this represents the best understood case but emphasize that we believe such combined action can explain chromosome folding more generally.

In interphase, along the length of each chromosome the epigenome alternates forming a sequence of chromatin domains of

different types. The process of (sub-) compartmentalization leads to spatial clustering of loci of similar types, through an affinity-driven process. This process naturally produces a stochastic assembly at the megabase-to-whole-chromosome scale. Additional constraints are imposed through tethering loci to the nuclear periphery, the nucleolus, speckles, etc. In addition, highly dynamic cohesin-mediated loop extrusion will bring loci together at the scale of up to hundreds of kilobases. All of these are acting on a chromatin fiber that is subject to topological constraints. As described above, cohesin extrusion patterns across the genome are guided by the presence of active enhancers that can facilitate cohesin loading, CTCF-bound sites that can block extrusion, and sites where cohesin is unloaded (e.g., downstream of active genes). These *cis*-elements determine a cohesin “traffic pattern”^{183,186} that produces, over the cell population, a range of structural features observed by Hi-C: formation of contiguous domains of enriched extrusion-dependent chromatin contacts (TADs) bounded by nearby CTCF sites; transient loops between convergent CTCF sites, enriched contacts between CTCF sites, and flanking domains (stripes or flares in Hi-C maps); and some enhancer-promoter interactions facilitated by loop extrusion and also by affinity-driven interactions (see below).

There is important interplay between different folding mechanisms. This is perhaps best exemplified by the interaction between compartmentalization and loop extrusion.²³⁷ Loop extrusion can extend to hundreds of kilobases and can cross from one sub-compartment domain into another, thereby bringing together loci of different chromatin states that would otherwise tend to spatially segregate. This affects not just directly adjacent domains, but the increased mixing of chromatin also appears to lead to mixing of domains at larger scale, e.g., interactions between compartment domains separated by large genomic distances or even located on different chromosomes. In effect, loop extrusion makes different sub-compartments segregate less than they otherwise would.

Simulations suggest that these are not only extruded loops but the whole active process of extrusion itself that weakens compartmentalization.²³⁷ This effect is particularly clearly visible in experiments where loop extrusion is abolished through rapid depletion of cohesin, e.g., using degron approaches to inducibly degrade subunits of the cohesin complex.^{214,238} In such experiments, compartmentalization is more pronounced, i.e., compartments segregate by type more strongly. In addition, smaller compartment domains emerged that in control cells appeared subsumed by the flanking domains in a cohesin-dependent manner. The compartment pattern seen in cohesin-depleted cells correlates better with the epigenome profile, again showing that loop extrusion interferes with the natural tendency for domains to compartmentalize via intrinsic affinity-driven processes.

While tethering of loci from the B compartment to the periphery is not directly driving compartmentalization itself,¹⁰⁴ it does determine which B domains interact with which other B domains on other chromosomes, observed with Hi-C.²³⁹ In the absence of such tethering, e.g., in the inverted nuclei of rod cells, the process of compartmentalization is unaffected, but the pattern in interchromosomal interactions between B compartment domains is altered.

Finally, even though topological entanglements along and between chromosomes appear to be rare in interphase (in eukaryotes), this does not mean that topological transitions do not play a role in modulating interphase chromosome folding in eukaryotes. For instance, the increased compartmentalization observed upon acute depletion of cohesin is partly dependent on topoisomerase II activity.²⁰⁸ Any real-time changes in compartmentalization may involve movement of loci, which may be facilitated by allowing topoisomerase II-dependent strand passage in general.

THE SAME MECHANISMS CAN PRODUCE DIFFERENT FOLDED STATES

In multicellular organisms, during interphase different cell types express different genes through differential activity of *cis*-regulatory elements, different patterns of histone modification, and DNA methylation. Given that affinity-driven compartmentalization as well as cohesin-mediated loop extrusion are directly guided and regulated by these features and *cis*-elements (above), the way the genome is folded in different cell types is different. However, although different loci will be clustered together or looped, the general folding principles are the same: affinities between sub-compartments will drive their spatial clustering, and loop extrusion will occur throughout the genome with cohesin being recruited, unloaded, and blocked at *cis*-elements active in that cell type.

In contrast, chromosome organization can appear very different in different species and kingdoms (e.g., prokaryotes vs. eukaryotes), and across the mitotic and meiotic cell cycles, suggesting the possibility that in these cases, very different folding principles and mechanisms may be at work. A key insight from extensive studies over the last decade on many different species, and with cells that synchronously progress through the cell cycle, has been that in all cases folding is driven by the same small set of mechanisms described above. The reason this is possible is that these mechanisms, and especially the process of loop extrusion, are particularly malleable and can be regulated in many different ways, resulting in a variety of chromosome architectures.

Below we provide examples of how differential deployment of loop extrusion, compartmentalization, tethering, and topological entanglements can give rise to a large diversity of structures seen throughout the cell cycle, and even across kingdoms.

Interphase vs. mitosis

The dramatic changes in chromosome morphology during the cell cycle serve as an excellent example of how cells can fold, unfold, and refold their genomes to accommodate gene expression in interphase and accurate chromosome segregation during mitosis (Figures 3B and 4B). As originally proposed based on extensive microscopy studies,^{30,133} biochemical and imaging experiments,^{141,160,240} and later genomic (5C and Hi-C) studies and polymer modeling,^{15,16,24} we now understand that by late prometaphase each sister chromatid is folded as a compressed array of consecutive loops. These loops are formed by condensin complexes: condensin II initially generates relatively large (400-kb to 1-Mb) loops in prophase, and during prometaphase,

condensin I then splits these in smaller (100-kb) loops.¹⁶ This generates a nested arrangement of loops. In contrast to interphase, where many loops are positioned at reproducible sites (e.g., CTCF sites), positioned loops are not observed in mitosis. The array of loops then acquires a helical organization. This helical organization requires condensin II and is irregular. Perversions, where the handedness of the helical turns alternates every half turn, have been observed as well, and these have been linked to the presence of connections between sister chromatids.²⁴¹ At the same time chromatin condenses through global reduction in histone acetylation, leading to general affinity-driven locus-locus interactions.²⁴² In Hi-C studies, no or only very weak A or B compartments have been observed.

The organization of mitotic chromosomes appears very distinct from interphase chromosomes described above. However, both states are driven by mechanistically similar loop extrusion processes and affinity-driven locus-locus interactions. What makes the structures distinct is the way these mechanisms are implemented.

First, in interphase, cohesin is the main loop-extruding complex, whereas in mitosis two types of condensin complexes act. This simple switch in extruder complexes explains much of the difference in interphase and mitotic chromosome folding. All complexes are estimated to extrude DNA at similar speeds (1–2 kb/s *in vitro*¹⁵⁶ and *in vivo*²⁴). However, cohesin has a relatively short residence time on chromatin (5–20 min), and therefore interphase loops are relatively sparse, short-lived, and dynamic. In contrast, condensin II complexes appear to rarely or never dissociate during mitosis,²⁴³ and therefore they can extrude larger and more stable loops. This also explains why in interphase only a fraction of DNA (~60%^{12,149}) is extruded in loops at any given time, while by prometaphase, almost the entire genome is extruded and contained within condensin loops (Figure 3B).

Second, cohesin and condensin differ in how they resolve encounters with other complexes and proteins while they extrude chromatin (above; Figure 4). Cohesin is blocked at CTCF-bound sites in a directional manner, leading to positioned and more stable loops between pairs of convergent CTCF sites that can be cell-type specific. Condensins, however, do not get blocked by CTCF²⁴⁴ or any other complex as far as we know, and therefore they do not form positioned loops. Interestingly, during mitosis in living cells, condensins do not appear to bypass one another *in vivo* (Figure 4), leading to consecutive rather than overlapping loops.^{15,16,24}

We note that the clear separation of action of cohesins and condensins during interphase and mitosis, respectively described above for vertebrates, is not always so clear. In *Drosophila*, condensin II plays roles in chromosome folding in interphase, and in *C. elegans*, condensin I contributes to folding the interphase genome.²⁴⁵ In budding yeast, cohesin extrudes loops during mitosis.²¹⁶

Third, cell-type-specific affinity-driven compartmentalization is a major feature of interphase chromosomes but is absent within mitotic chromosomes. This has been puzzling because the patterns of histone modifications along chromosomes that correlate strongly with (sub-) compartments are largely preserved throughout the cell cycle. There are several possible explanations.

First, it is possible that the factors that in interphase mediate the affinity between compartment domains are inactivated. A good example is the family of HP1 proteins. These proteins can bridge loci containing the H3K9me3 modification. In mitosis, the residue immediately adjacent to K9 (H3S10) becomes phosphorylated. Histone tails carrying both modifications, H3K9me3 and H3S10P, cannot be bound by HP1 proteins.²⁴⁶ Given that histone tails become massively phosphorylated during mitosis, it is possible that many other bridging factors cannot bind, and thus affinity-driven compartmentalization will be prevented. An alternative, or additional explanation comes from a very recent study that showed that when condensins are depleted while cells are arrested in prometaphase, some form of compartmentalization is observed.¹¹⁵ This result suggests that the factors and mechanisms for compartmentalization are active during mitosis but are somehow overruled by the condensin-driven loop array formation, similar to how in interphase cohesin-mediated loop extrusion counteracts compartmentalization.

Fourth, tethering of loci to the nuclear periphery, nucleoli, and speckles dominate interphase nuclear organization. During mitosis these structures are disassembled, and as a result the genome becomes untethered so that free rod-shaped mitotic chromosomes can form.

Fifth, topological entanglements within each chromosome are rare in interphase, but self-entanglements within individual sister chromatids are abundant in mitosis (above). This difference can at least in part be explained simply by the fact that topoisomerase IIa activity is high in mitosis, which together with a condensed and compacted chromatin state will drive the chromosomes toward becoming self-entangled. Polymer theory predicts that such change in topological state will facilitate rapid compaction, as would be required during prometaphase.⁷⁸ However, more active processes driving self-entanglements may also be at work.

Long vs. short mitotic chromosomes

In vitro, condensin complexes can bypass rather larger objects,¹⁷² including other condensin complexes.¹⁷¹ *In vivo*, during mitosis it appears that such bypassing of condensins is rare, at least for condensin II in vertebrates.^{16,24} As a result, condensin II complexes extrude loops till they encounter one another and then stop so that a tightly spaced consecutive loop array is formed. This also strictly requires two-sided extrusion activity by condensin II,²⁴⁷ as observed in single-molecule experiments.^{164,165} The size of these loops, assuming condensin II does not turn over, will be determined by how many condensins are recruited to chromatin.¹⁵⁸ When many condensins are recruited, loops will tend to be small and mitotic chromatids will be relatively long and narrow. When fewer condensins are recruited, loops will on average be larger, and mitotic chromatids will be shorter and wider. Thus, in theory, the overall dimensions of mitotic chromosomes can be regulated simply by regulating condensin recruitment.

Interestingly, mitotic chromosomes can have very different dimensions when compared between species, or even within a species but at different stages of development. For instance, when mitotic chromosome dimensions are compared for human and mouse cells, it was observed that they differ in the amount of DNA that is packed per micron length of chromosome.²⁴⁴ The

difference was correlated with different loop sizes: in mouse cells, the mitotic loops are considerably larger than in human cells (1 Mb vs. 400 kb), suggesting fewer condensins are recruited per megabase in mouse cells as compared with human cells. Intriguingly, the difference may be related to the fact that mouse chromosomes are all acrocentric, and thus the longest chromosome arm in the mouse genome is much longer than the longest arm in the human genome. Increasing loop size through regulating condensin recruitment genome-wide may ensure that even the longest chromosomes are short enough to facilitate their segregation during anaphase.

A similar adaptive scaling of mitotic chromosome dimensions appears to occur during *Xenopus* development.²⁴⁸ During early cleavage stages of development, the cells are very large, and mitotic chromosomes are relatively long. At later stages of development, when cells are much smaller, mitotic chromosomes become increasingly short. Again, analysis of loop sizes showed that the difference is due to the formation of small loops in early stages and larger loops at later stages. Differential recruitment of condensin complexes would explain this phenomenon. Interestingly, factors on the chromatin in differentiated cells appear to reduce condensin loading. One such factor could be histone H1.8. *In vitro* reconstitution experiments showed that in *Xenopus* egg extracts, depletion of H1.8 resulted in increased condensin recruitment, longer chromosomes, and smaller loops.²⁴⁹

These examples show that by simply regulating the recruitment of loop-extruding factors, the same process of loop extrusion can produce mitotic chromosomes of distinct dimensions. This makes mitotic chromosome architecture adaptable to ensure condition-appropriate scaling of chromosome arm length.

Mitosis vs. meiosis

Mitotic and meiotic chromosomes are both folded as arrays of loops to form rod-shaped compacted chromatids. While transcription ceases during mitosis and compartments become undetectable, during meiotic prophase I, transcription continues and a form of compartmentalization remains present.^{250,251} Another key difference is how sister chromatids are arranged with respect to each other: in mitosis, by prometaphase, sister chromatids are connected through cohesin-mediated connections within their loops. Microscopically, this can be deduced from the fact that cohesin complexes are localized in between the masses of each sister chromatid and away from the condensin complexes that are located at the bases of the loops in the center of each chromatid.^{24,252} In contrast, during meiotic prophase, sisters are cohesed at the bases of the loops.²⁵³ We recently proposed that the mitotic arrangement could arise naturally when actively extruding condensins step over cohesin complexes that hold sister chromatids together (so-called cohesive cohesin complexes).²⁴ This will result in cohesive cohesin being localized inside condensin-mediated loops. Modeling showed that when condensin, or any other loop-extruding complex (cohesin likely in meiosis), cannot bypass cohesive cohesins, the extruding complexes and cohesive cohesins will both localize at the base of the loops, a scenario that may be present during meiotic prophase. It is therefore possible that by simply modulating the ability to bypass cohesive cohesins, one can obtain either the mitotic or meiotic arrangement of sister chromatids

(Figure 4B). Consistent with this proposal is that during meiosis, cohesin plays a significant role in loop array formation, and a recent study showed that (mitotic) cohesin cannot bypass cohesive cohesin.¹⁸⁴ CTCF, which remains chromatin bound during meiotic prophase and is also found at the conjoined axes of sister chromatids, may also contribute to this arrangement of cohesin-mediated sister loops.²⁵⁴

Finally, it is noteworthy that at early prophase stages in mitosis in human cells, sister chromatids are also transiently connected at their loop bases.²⁵⁵ Possibly condensins initially stall at cohesive cohesin complexes and only later bypass. Clearly, how SMC complexes resolve encounters between them during interphase, mitosis, and meiosis can lead to very distinct chromosome conformations at the macro scale. Several of such rules of engagement have now been described,²⁴ but surely additional ones remain to be discovered.

Across the tree of life: Prokaryotes vs. eukaryotes

Although bacterial nucleoids and eukaryotic chromosomes appear very different in size and conformation, similar processes fold these genomes. Such similarities were already recognized and reviewed a number of years ago.²⁵⁶ Loop extrusion by SMC-like complexes act on bacterial chromosomes, and as in eukaryotic chromosomes, *cis*-elements can determine where these complexes load and where they are blocked (for review, see Yáñez-Cuna and Koszu²⁵⁷). For instance, in *B. subtilis*, the ParS sites at the centromere recruits SMC complexes that then start to extrude DNA bi-directionally, leading to co-alignment of the arms of the chromosomes.¹⁷⁵ In *E. coli*, SMC-like complexes extrude DNA within each arm, thereby condensing the nucleoid. Interestingly, in *B. subtilis*, with engineered arrangement of ParS sites complicated patterns of folding have been observed that can be explained when SMC complexes can bypass one another.¹⁸⁵ Such bypassing can be critical to avoid traffic jams between SMCs loaded at nine native proximal ParS sites, providing another example of a “rule of engagement” whereby resolution of molecular encounters between extruding complexes can determine folding of entire genomes.

In bacteria, topological features appear to play a much more dominant role in chromosome folding than in interphase in eukaryotes. Supercoiling will compact the nucleoid and is determined by transcription and replication but also directly by enzymes such as gyrase that introduce positive supercoiling. Interestingly, chromosomal interaction domains (CIDs) have been observed along the *C. crescentus* and *B. subtilis* chromosomes that in Hi-C resembles TADs.¹⁷⁶ CIDs, however, could be formed in an SMC-independent manner as dense arrays of plectonemes that are separated by plectoneme-free regions at highly expressed genes.

Finally, even though conventional compartmentalization is not observed, some form of affinity-driven clustering of loci can occur in bacteria. For instance, nucleoid-associated proteins, such as HU and H-NS, can act as bridging factors condensing chromosomal domains.²⁵⁸

Across the tree of life: More variations of folding mechanisms, and possibly additional mechanisms?

In a recent study, chromosome folding for 24 eukaryotic species from across the tree of life was studied by Hi-C.⁵¹ These included

several vertebrate classes and animal phyla, plants, and fungi. Two main types of folding architectures were described: one type is defined by a Rabl-like organization with centromeres clustered, telomeres clustered, and/or chromosome arms being aligned to each other from centromere to telomere; the second type lacks these features but has chromosome territories. Interestingly, a single factor, the presence or absence of condensin II, defines whether the first type or the second type is formed. The authors proposed a model where condensin II mediates length-wide compaction during mitosis, which then facilitates chromosome territory formation in the next G1, while preventing centromere and telomere clustering. This study shows how genome-wide chromosome-folding patterns in eukaryotes can be altered by simply turning one extrusion complex on or off.

These results show that studying chromosome folding in a range of species can be fruitful for gaining a better understanding of the basic chromosome folding mechanisms discussed here. Through such an approach, we can discover additional ways in which these conserved mechanisms can be regulated and implemented. Possibly new variants of the basic machinery, e.g., additional SMC complexes, can be discovered. Finally, it is possible that entirely new and yet to be discovered mechanisms of folding chromosomes remain to be discovered in groups of species with highly divergent chromosome conformations.

We envision two ways to select groups of organisms for such evolutionary studies. First, one can study groups of organisms that contain distinct variants of the conserved machineries for chromosome folding. An example are the two major groups of archaea, the euryarchaea and crenarchaea. These single-cell organisms differ in how they organize chromatin and have been studied only recently using 3C-based assays. Interestingly, both archaeal groups express SMC-related proteins, and these proteins may play roles in chromosome folding.²⁵⁹ In most cases in euryarchaea, these complexes are clearly related to condensins. Intriguingly, the crenarchaea appear to have lost the condensin-like SMC complex and instead have acquired a poorly characterized SMC-like complex called coalescin.²⁶⁰ Hi-C studies show that coalescin plays roles in chromosome folding and compartmentalization. The fact that an SMC-related complex may be involved in compartmentalization may point to a new role for an SMC complex, and it highlights how the study of divergent species can provide opportunities to discover new roles or new ways to employ these otherwise conserved machineries.

In a second approach, one can select species that display chromosome conformations that appear particularly different from any other group of species. One example is the dinoflagellates. Dinoflagellates are single-cell eukaryotes with very large genomes (up to hundreds of gigabases), which do not wrap the bulk of their genome around nucleosomes. Macroscopically, dinoflagellate chromosomes appear very distinct from any other group^{261,262}: the chromosomes are permanently condensed through the cell cycle, and they have optical properties that suggest a liquid crystalline arrangement of chromatin fibers within them. Recent Hi-C studies show that the chromosomes are composed of structural domains, resembling TADs and CIDs, each of which contains a pair of divergently transcribed gene arrays.^{7,8} Very little is known about the mechanism of chromosome

folding in these organisms. They express condensin- and cohesin-like complexes, and therefore it is possible that they represent yet another example where new ways have evolved to employ these conserved folding machines. On the other hand, given that their chromosomes appear so different from any other group, it is also possible that new mechanisms to fold chromosomes have emerged in this lineage.

STRUCTURE-FUNCTION RELATIONSHIPS

Higher-order chromosome folding in eukaryotes is linked to genomic functions, including for instance, chromosome compaction, segregation, and regulation of transcription. However, despite extensive efforts, it has proven difficult to demonstrate that higher-order folding has consistent, conserved, and genome-wide consequences for gene expression. This is likely because the primary function of chromosome folding is not gene regulation but instead is for cells to manage long DNA molecules to ensure their replication, compaction, segregation, and subsequent decompaction. Consistent with this, the proteins involved in chromosome compaction, especially the factors that perform loop extrusion appear conserved in all organisms. We propose that once mechanisms for folding and unfolding genomes are in place, these same mechanisms, e.g., loop extrusion and affinity-driven clustering of loci, are subsequently co-opted for roles in additional processes, including long-range regulation of genes by distal regulatory elements, etc. (see below). Given that these latter functions are secondary, and possibly ad hoc, chromosome folding will not necessarily have consistent, conserved, and genome-wide roles for regulating expression of all genes and in different organisms. Such roles of chromosome-folding processes in gene regulation and, broadly, in epigenetic mechanisms have started to emerge in disparate biological systems, with universal effects yet to be discovered.

Functional roles for mitotic chromosome compaction

The most obvious functional role of chromosome folding is related to chromosome duplication and segregation. In large vertebrate genomes, loci replicate at different times during S-phase. This phenomenon shows a clear connection with chromosome folding: DNA replication timing is strongly correlated with compartmentalization,²⁶³ and when replication timing is disrupted or altered, this can lead to changes in compartmentalization.²⁶⁴ After replication, sister chromatids are to a significant level topologically intertwined. These interlinks need to be resolved to facilitate their segregation during anaphase. In addition, each sister chromatid needs to compact into mitotic chromatids. In eukaryotes this involves the formation of arrays of loops mostly by the condensin complexes (above). These loops can become topologically interlinked (i.e., mitotic chromatids can become self-entangled). These two processes are likely mechanistically linked as loop extrusion in *cis*, in the presence of topoisomerase II enzymes, and will automatically drive the sister chromatids to become unlinked while they are still held together by cohesive cohesin complexes.¹⁴⁸ The function of this elaborate process of folding, self-entangling, and unlinking sister chromatids is to facilitate chromosome segregation to

daughter cells. Any functional role of self-entanglement is less understood. It has been shown, through micromechanical measurements of single isolated mitotic chromosomes, that human mitotic chromosomes are self-entangled and that these entanglements contribute to mechanical rigidity of chromosomes.²¹² Such rigidity may be important for chromosome segregation to counteract spindle forces.

Roles of chromosome folding in controlling gene expression

In eukaryotic cells, genes can be regulated by enhancers that are located up to many hundreds of kilobases from their promoter. A long-standing model has been that the spatial folding of chromosomes would allow enhancers to loop to gene promoters and through physical interactions between complexes bound to the enhancer and the promoter and possibly, through factors bridging them such as the Mediator complex, would activate transcription.^{265,266} Other mechanisms have also been put forth, such as models where factors recruited at an enhancer can somehow travel along chromatin over considerable distances and in that way reach target promoters that are then activated without needing a direct physical interaction between these elements ("tracking models"). In the latter case, chromosome folding may not be critical for long-range gene regulation. It now appears multiple mechanisms may contribute.

One of the first applications of 3C-based assays had been to determine whether looping interactions between genes and their distal regulatory elements occur. In a very early study, it was found that the locus control region (LCR) of the mouse beta-globin locus physically touches a target beta-globin gene only in cell types where that gene is expressed.²⁶⁷ Further work showed that when different beta-globin genes become active during development, the LCR switches its long-range interactions accordingly.²⁶⁸ This locus remains one of the best studied examples of long-range interactions in relation to gene expression. In a demonstration of the importance of physical interactions between enhancers and promoters, it was shown that direct tethering of the distal LCR to the target gene is sufficient for activation of the gene.²⁶⁹

Since then, numerous studies have detected many more long-range promoter-enhancer interactions, e.g., in single gene studies for the alpha-globin locus,¹²⁹ in higher throughput studies for hundreds of genes throughout numerous targeted regions of the human genome (5C²⁷⁰), or in genome-wide analyses using targeted approaches (Capture-C,²⁷¹ and ChiaPET⁵⁹). From these studies, one would conclude that the looping model is firmly established. However, other lines of experimentation produced observations that were at first glance not consistent with the "activation by physical contact" model for long-range control of gene expression. First, imaging-based studies showed that 3D spatial distances between enhancers and promoters do not correlate well with gene activation, neither in live cells in real time nor over cell populations (reviewed by Chen et al.²⁷²). Furthermore, a relatively small (~2- to 3-fold) increase in the contact frequency within a TAD results in a much greater activation of transcription. This lack of correlation between contacts, or close proximity, and gene activation may appear to be inconsistent with a mechanistic role of looping contacts. Sec-

ond, even when enhancers and promoters have been observed in close spatial proximity, the distance that separates them was relatively large, i.e., 300 nm.²⁷³

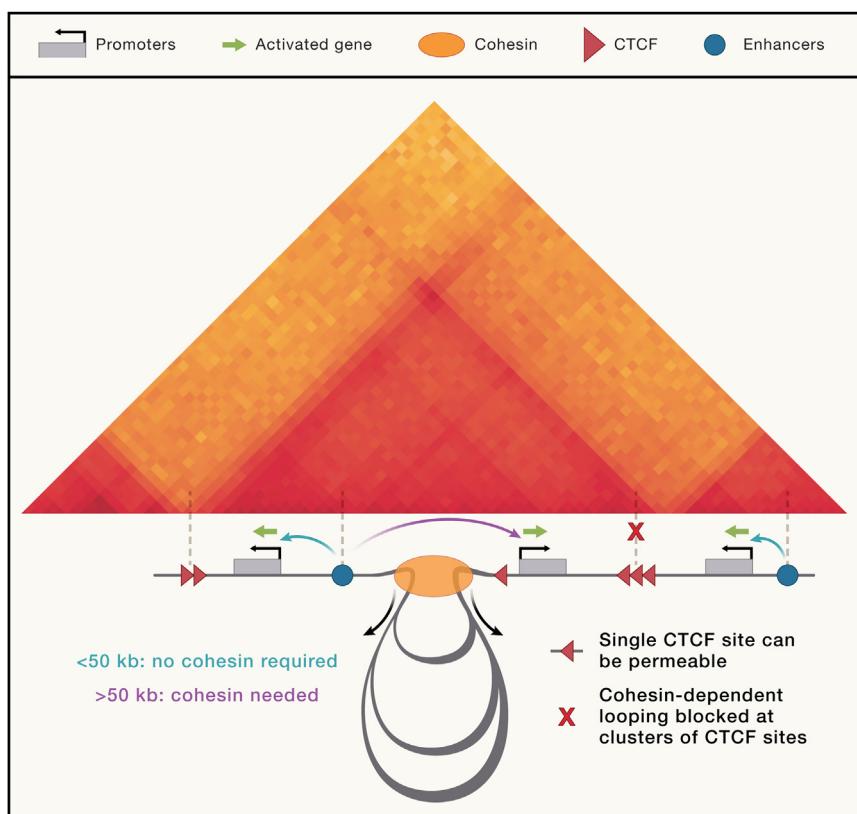
Several new insights, experimental approaches and models, are now attempting to unify these observations. First, the discovery that transcription complexes can form condensates that can be several hundreds of nanometers in diameter suggests that such condensates can potentially mediate connections between enhancer and promoters.²⁷³ By imaging, the enhancer and promoter can then appear not to be directly touching, yet 3C-based assays that employ formaldehyde crosslinking may still detect these contacts as looping interactions.

Second, several models have been proposed to explain the lack of simple correlation between the frequency of enhancer-promoter contacts (measured by Hi-C and microscopy) and transcription from the target promoter.^{274,275} The idea of these models is that transient and rare enhancer-promoter interactions result in the incremental but cumulative changes in the multi-state promoter, with only the final state resulting in transcription. These changes may, for example, reflect the accumulation of marks at the promoter and enhancer. Importantly, such multi-state models show a sigmoidal response to contact frequency with small changes in contact frequency resulting in large changes in transcription. When a critical level of marks is delivered, the promoter will become active. These models of multi-state promoters await validation by experimental live-cell dynamic measurements of distance and transcription at high temporal and spatial resolutions.

What is the mechanism by which an enhancer can loop to target promoters? One attractive proposal is that cohesin-mediated loop extrusion actively brings elements together. The observation that transcriptional elements, such as enhancers, promoters, insulators (CTCF sites), and 3' ends of active genes, all play roles in recruiting, pausing, blocking, and unloading cohesin, respectively, already suggests links between transcriptional control and cohesin-mediated loop extrusion.^{183,186} However, acute depletion of cohesin was found to have little immediate effect on transcription.^{154,214} Most recent studies, however, demonstrated that the loss of cohesin activity in postmitotic cells has a profound effect on cell and organism physiology, such as neuronal maturation²⁷⁶ and differentiation and response to activation by dendritic cells of the innate immune system.²⁷⁷

After years of conflicting observations, a unified view is now emerging on how cohesin plays a role in long-range gene regulation (Figure 6). In this view, cohesin can be loaded at random positions, with some preference for *cis*-elements such as enhancers. These complexes can then extrude loops and through this process reach distal target promoters. This can lead to detectable enhancer-promoter loops in 3C-based assays. However, in such assays, these interactions appear weak, suggesting these are either rare or very transient contacts. As stated above, repeated transient interactions may be sufficient to trigger changes in promoter states that will become active after several interactions.

Another factor is that enhancer-promoter communication may require a different type of interaction than contacts captured by 3C-based assays or microscopy. For example, functional communication may require close interactions between



molecular complexes, i.e., ~5–10 nm separation, or a prolonged contact, while 3C-based assays and microscopy detect mere proximity of 100–300 nm. It remains to be studied how proximity—spatial, cohesin mediated, or affinity mediated—may translate into functional communications. We note that it is also possible that cohesin will travel in *cis* from the enhancer to the promoter carrying transcription-activating complexes picked up at the enhancer, which will be delivered to the promoter, and this tracking process would not necessarily involve physical promoter-enhancer interactions.

The observation that acute depletion of cohesin has little direct effect on transcription genome-wide is now understood to be at least in part due to most promoters being regulated by enhancers that are located relatively close to the start site (<50 kb). Several studies have now shown that enhancers can interact with promoters over such short genomic distances without the assistance of cohesin,^{186,278,279} while cohesin-mediated extrusion becomes important only for enhancers to reach target genes located >50 kb away. Enhancer-promoter interactions are also likely assisted by affinity-based interactions, e.g., like compartmentalization, without a need for cohesin. The frequency with which affinity-based interactions occur in the population will be inversely related to the genomic distance between them.¹²⁸ It appears that when they are less than 50 kb apart, the random dynamics and conformation of chromatin fibers ensure that they interact sufficiently frequently to enable gene activation, whereas enhancers located farther away only contact their targets when loci are actively

brought together through extrusion. Direct visualization and models of enhancer-promoter interactions and resulting transcription (e.g., Brückner et al.²²⁷ and Chen et al.²⁸⁰) will be instrumental in elucidating this interplay of extrusion-mediated and spatial contacts in gene regulation.

If cohesin assists enhancers to reach, and activate, genes that can be located

hundreds of kilobases away in the linear genome, how is specificity controlled? CTCF-bound elements, when located in between genes and enhancers, can block both enhancer action toward a promoter and block loop extrusion in an orientation-dependent manner (see above). This has led to models that pairs of convergent CTCF sites form the boundaries of structural domains.²⁸¹ An enhancer located within a domain would contact promoters in the same domain, but interactions with promoters outside the domain would be less frequent (although not completely inhibited).

Hox loci in mouse provide particularly well-studied and well-characterized examples of how CTCF boundaries prevent or allow enhancer-promoter interactions (see Rekaik and Duboule²⁸² for a recent review). These clusters of multiple hox genes are regulated by the progressive movement of CTCF boundaries in cells positioned along the posterior-anterior body axis, allowing progressive activation of the hox genes in order of their location along the genomic locus. Another example is the regulation of the protocadherin clusters where regulated stochastic CTCF binding to different protocadherins promoters, in combination of cohesin-mediated loop extrusion, defines cell-to-cell variation in expression of different variants of protocadherins (Kiefer et al.²⁸³ and see Canzio and Maniatis²⁸⁴ for a review).

The model also predicts that loss of a domain boundary, e.g., in disease through deletion of CTCF-binding sites, would allow for the formation of new enhancer-promoter contacts that in

normal cells would rarely occur. There are multiple examples where this prediction is fulfilled. For instance, in T cell acute lymphoblastic leukemia recurrent deletions around the TAL1 and LMO2 oncogenes result in loss of CTCF-bound domain boundaries.²⁸¹ Deletion of these boundaries exposes these genes to enhancers in adjacent domains that they normally rarely interact with. Inappropriate DNA methylation of CTCF-binding sites can also prevent CTCF binding and in that way prevent domain boundary formation. An example is DNA hypermethylation observed in gastrointestinal stromal tumors.^{285,286} This leads to inactivation of CTCF-dependent domain boundaries and formation of new enhancer-promoter interactions, including at the FGF8 locus, which in turn drives tumorigenesis. Thus, loop extrusion can facilitate very long-range enhancer-promoter interactions in general, and CTCF-dependent blocking of extrusion can impose specificity by preventing some interactions.

Long-range gene regulation is clearly complex and driven by a multitude of processes: for some cases, loop extrusion is critical, for others it is not. CTCF can prevent long-range interactions between enhancers and promoters, but sometimes affinity-driven interactions can override this. Not all CTCF-bound sites act in the same manner, possibly dependent on how many CTCF sites are near one another, their orientation, the presence of other DNA-binding sites, and other factors.

Other processes that have co-opted the loop extrusion process

Loop extrusion has been co-opted by other more specialized genomic processes. We highlight one example.

Immunoglobulin locus rearrangement

B and T cells can express an enormous diversity of antigen receptor molecules: antibodies in B cells and T cell receptors in T cells. This diversity is the result of rearrangements of immunoglobulin and T cell receptor loci. For instance, the immunoglobulin H (IgH) locus is composed of three sets of loci: V, D, and J segments. There are 13 V segments, 4 D segments and 113 J segments. In a given B cell, the locus recombines so that a single V region gets placed next to a single D segment, and then 1 J segment is selected to be recombined adjacent to the D segment. This progressive recombination occurs only on one allele. As a result, a given B cell will express only IgH polypeptide that is encoded by one V, D, and J segment. Wood and Tonegawa proposed, already in 1983,¹³⁸ that loop extrusion could play a role in bringing V, D, and J segments, spread out over a large 2.5-Mb region, in close spatial proximity for their subsequent recombination. Their argument was based on the fact that all V, D, and J segments are in the same orientation. A tracking model was proposed where a complex would track along the locus and bring distal loci together for subsequent recombination.

There is now strong evidence that cohesin plays a key role in immunoglobulin locus rearrangements (reviewed more comprehensively by Peters²⁸⁷ and Zhang et al.²⁸⁸). First, IgH recombination is dependent on cohesin. Depletion of cohesin subunits inhibits V(D)J recombination.²⁸⁹ Second, CTCF sites are located throughout the locus, and they occur in convergent orientations: CTCF sites throughout the section of the locus encoding J segments are oriented toward the 3' end of the locus, while CTCF

sites at the 3' end of the locus are oriented toward the 5' end containing the J segments. This convergent orientation is strongly predictive of a role of cohesin-mediated loop extrusion. Third, inversion of large sections of the locus changes the selection of segments due to inversion of CTCF sites.²⁹⁰ Fourth, deletion of individual CTCF sites, or ectopic insertion of CTCF sites, changes the selection probability of the adjacent segment in ways that are consistent with roles of CTCF in blocking loop extrusion.²⁹¹ Fifth, in B cells expression of WAPL is reduced through Pax5-mediated repression of the WAPL gene, and this increases the residence time of cohesin on chromatin genome-wide. As a result, cohesin-mediated loops can become larger, and this facilitates the very long-range looping that is required in the IgH locus to select the most distal J segments for recombination.²⁹²

Epigenetic memory: A functional role of compartmentalization?

A recent study has put forward a hypothesis that spatial segregation and higher heterochromatin density can be key for the maintenance of repressive heterochromatin marks and broadly "epigenetic memory."²⁹³ Since marked histones are constantly lost and replaced with non-marked ones, particularly during replications, the patterns of histone marks need to be restored by the activity of reader-writer histone methyltransferases. Models show that such activity could lead to either uncontrolled spreading or to complete loss of marks. Yet creating dense and spatially segregated heterochromatin by virtue of making heterochromatic regions attract each other can put this process under control, with another key ingredient being the limited amount of the reader-writer enzyme. Polymer models show that such a mechanism can be self-sustained in maintaining epigenetic memory, i.e., preserving an initially deposited pattern of marks for up to hundreds of cell divisions. Importantly, this mechanism suggests that the memory is preserved in the form of spatial folding (segregation and density) of chromosomes during interphase and in the form of the pattern of marks when chromosomes are refolded into mitosis and back into the interphase. Perturbing chromosome folding experimentally would test this hypothesis. Broadly, this study suggests that one of the functions of compartmentalization may be to help maintain the pattern of epigenetic marks.

FUTURE PERSPECTIVE

We described the biophysical and molecular mechanisms that drive chromosome folding and that allow cells to solve their chromosome-folding problem. Many open questions remain to be answered about how these mechanisms are regulated and how they influence one another. Going forward, a better understanding of the molecular mediators of compartmentalization and its function in genomic activities is needed. How the basic process of loop extrusion works at the nanometer scale is increasingly clear, but we are just beginning to understand how this process, with interplay with other mechanisms, leads to a variety of chromosome conformations at the micro-meter scale. Regulation of loop extrusion likely involves cell-cycle-dependent, condition-dependent, and species-dependent

expression of extrusion complexes, their targeted and non-targeted recruitment, and their posttranslational modifications. Interactions between extruders and with other DNA-bound complexes must occur frequently, and how they are resolved largely determines the conformation of whole chromosomes. Such rules of engagements are only now starting to be defined, but they will likely be key to our understanding of how activities at the nanometer scale lead to differential folding at the micrometer scale.

Much has been learned from the study of chromosome folding and changes in folding throughout the cell cycle. We anticipate that additional insights will come from studies across the tree of life, where different organisms may have evolved additional solutions to the chromosome folding problem. On the one hand, it is possible that the same mechanisms fold chromosomes in all species but that these mechanisms are employed in different ways to give rise to chromosomes that can look very different. In that case, studying chromosome folding in different species will provide deeper insights into such universal mechanisms. On the other hand, it is possible that entirely different mechanisms remain to be discovered in highly divergent species. The coming 50 years promise to be as exciting for the chromosome folding field as the last few decades have been.

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DECLARATION OF INTERESTS

J.D. is a member of the scientific advisory board of Arima Genomics, San Diego, CA, USA, and Omega Therapeutics, Cambridge, MA, USA. J.D. is listed as co-inventor of patents describing the 5C and Hi-C technologies.

REFERENCES

- Ris, H., and Kubai, D.F. (1970). Chromosome structure. *Annu. Rev. Genet.* 4, 263–294. <https://doi.org/10.1146/annurev.ge.04.120170.001403>.
- Thomas, C.A., Jr. (1971). The genetic organization of chromosomes. *Annu. Rev. Genet.* 5, 237–256. <https://doi.org/10.1146/annurev.ge.05.120171.001321>.
- Oudej, P., Gross-Bellard, M., and Chambon, P. (1975). Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* 4, 281–300. [https://doi.org/10.1016/0092-8674\(75\)90149-x](https://doi.org/10.1016/0092-8674(75)90149-x).
- Weintraub, H., Palter, K., and Van Lente, F. (1975). Histones H2a, H2b, H3, and H4 form a tetrameric complex in solutions of high salt. *Cell* 6, 85–110. [https://doi.org/10.1016/0092-8674\(75\)90077-x](https://doi.org/10.1016/0092-8674(75)90077-x).
- Ryder, O.A., and Smith, D.W. (1975). Properties of membrane-associated folded chromosomes of *E. coli* related to initiation and termination of DNA replication. *Cell* 4, 337–345. [https://doi.org/10.1016/0092-8674\(75\)90154-3](https://doi.org/10.1016/0092-8674(75)90154-3).
- Allen, J.R., Roberts, M., Loeblich, A.R., 3rd, and Klotz, L.C. (1975). Characterization of the DNA from the dinoflagellate *Cryptocodium cohnii* and implications for nuclear organization. *Cell* 6, 161–169. [https://doi.org/10.1016/0092-8674\(75\)90006-9](https://doi.org/10.1016/0092-8674(75)90006-9).
- Nand, A., Zhan, Y., Salazar, O.R., Aranda, M., Voolstra, C.R., and Dekker, J. (2021). Genetic and spatial organization of the unusual chromosomes of the dinoflagellate *Symbiodinium microadriaticum*. *Nat. Genet.* 53, 618–629. <https://doi.org/10.1038/s41588-021-00841-y>.
- Marinov, G.K., Trevino, A.E., Xiang, T., Kundaje, A., Grossman, A.R., and Greenleaf, W.J. (2021). Transcription-dependent domain-scale three-dimensional genome organization in the dinoflagellate *Breviolum minutum*. *Nat. Genet.* 53, 613–617. <https://doi.org/10.1038/s41588-021-00848-5>.
- Dekker, J. (2008). Mapping *in vivo* chromatin interactions in yeast suggests an extended chromatin fiber with regional variation in compaction. *J. Biol. Chem.* 283, 34532–34540. <https://doi.org/10.1074/jbc.M806479200>.
- Arbona, J.M., Herbert, S., Fabre, E., and Zimmer, C. (2017). Inferring the physical properties of yeast chromatin through Bayesian analysis of whole nucleus simulations. *Genome Biol.* 18, 81. <https://doi.org/10.1186/s13059-017-1199-x>.
- Dekker, J., and Mirny, L.A. (2016). The 3D genome as moderator of chromosomal communication. *Cell* 164, 1110–1121. <https://doi.org/10.1016/j.cell.2016.02.007>.
- Gabriele, M., Brandão, H.B., Grosse-Holz, S., Jha, A., Dailey, G.M., Catoggio, C., Hsieh, T.S., Mirny, L., Zechner, C., and Hansen, A.S. (2022). Dynamics of CTCF- and cohesin-mediated chromatin looping revealed by live-cell imaging. *Science* 376, 496–501. <https://doi.org/10.1126/science.abn6583>.
- Delbrück, M. (1954). On the Replication of Desoxyribonucleic Acid (DNA). *Proc. Natl. Acad. Sci. USA* 40, 783–788. <https://doi.org/10.1073/pnas.40.9.783>.
- Mirny, L.A. (2023). Chromosome and protein folding: in search for unified principles. *Curr. Opin. Struct. Biol.* 87, 102610. <https://doi.org/10.1016/j.sbi.2023.102610>.
- Naumova, N., Imakaev, M., Fudenberg, G., Zhan, Y., Lajoie, B.R., Mirny, L.A., and Dekker, J. (2013). Organization of the mitotic chromosome. *Science* 342, 948–953. <https://doi.org/10.1126/science.1236083>.
- Gibcus, J.H., Samejima, K., Goloborodko, A., Samejima, I., Naumova, N., Nuebler, J., Kanemaki, M.T., Xie, L., Paulson, J.R., Earnshaw, W.C., et al. (2018). A pathway for mitotic chromosome formation. *Science* 359, eaao6135. <https://doi.org/10.1126/science.aaq6135>.
- Shintomi, K., Takahashi, T.S., and Hirano, T. (2015). Reconstitution of mitotic chromatids with a minimum set of purified factors. *Nat. Cell Biol.* 17, 1014–1023. <https://doi.org/10.1038/ncb3187>.
- Kinoshita, K., and Hirano, T. (2017). Dynamic organization of mitotic chromosomes. *Curr. Opin. Cell Biol.* 46, 46–53. <https://doi.org/10.1016/j.celb.2017.01.006>.
- Swedlow, J.R., and Hirano, T. (2003). The making of the mitotic chromosome: modern insights into classical questions. *Mol. Cell* 11, 557–569. [https://doi.org/10.1016/s1097-2765\(03\)00103-5](https://doi.org/10.1016/s1097-2765(03)00103-5).
- Dekker, J. (2014). Two ways to fold the genome during the cell cycle: insights obtained with chromosome conformation capture. *Epigenetics Chromatin* 7, 25. <https://doi.org/10.1186/1756-8935-7-25>.
- Sood, V., and Misteli, T. (2022). The stochastic nature of genome organization and function. *Curr. Opin. Genet. Dev.* 72, 45–52. <https://doi.org/10.1016/j.gde.2021.10.004>.
- Misteli, T. (2020). The Self-Organizing Genome: Principles of Genome Architecture and Function. *Cell* 183, 28–45. <https://doi.org/10.1016/j.cell.2020.09.014>.
- Gibcus, J.H., and Dekker, J. (2013). The hierarchy of the 3D genome. *Mol. Cell* 49, 773–782. <https://doi.org/10.1016/j.molcel.2013.02.011>.
- Samejima, K., Gibcus, J.H., Abraham, S., Cisneros-Soberanis, F., Samejima, I., Beckett, A.J., Pučeková, N., Abad, M.A., Medina-Pritchard, B., Paulson, J.R., et al. (2024). Rules of engagement for condensins and

- cohesins guide mitotic chromosome formation. bioRxiv. <https://doi.org/10.1101/2024.04.18.590027>.
25. Abramo, K., Valton, A.L., Venev, S.V., Ozadam, H., Fox, A.N., and Dekker, J. (2019). A chromosome folding intermediate at the condensin-to-cohesin transition during telophase. *Nat. Cell Biol.* 21, 1393–1402. <https://doi.org/10.1038/s41556-019-0406-2>.
 26. Zhang, H., Emerson, D.J., Gilgenast, T.G., Titus, K.R., Lan, Y., Huang, P., Zhang, D., Wang, H., Keller, C.A., Giardine, B., et al. (2019). Chromatin structure dynamics during the mitosis-to-G1 phase transition. *Nature* 576, 158–162. <https://doi.org/10.1038/s41586-019-1778-y>.
 27. Pelham-Webb, B., Polyzos, A., Wojenski, L., Kloetgen, A., Li, J., Di Giammartino, D.C., Sakellaropoulos, T., Tsirigos, A., Core, L., and Apostolou, E. (2021). H3K27ac bookmarking promotes rapid post-mitotic activation of the pluripotent stem cell program without impacting 3D chromatin reorganization. *Mol. Cell* 81, 1732–1748.e8. <https://doi.org/10.1016/j.molcel.2021.02.032>.
 28. DuPraw, E.J. (1970). *DNA and Chromosomes* (Holt, Rinehart and Winston, Inc.).
 29. Manuelidis, L., and Chen, T.L. (1990). A Unified Model of eukaryotic chromosomes. *Cytometry* 11, 8–25. <https://doi.org/10.1002/cyto.990110104>.
 30. Marsden, M.P., and Laemmli, U.K. (1979). Metaphase chromosome structure: evidence for a radial loop model. *Cell* 17, 849–858. [https://doi.org/10.1016/0092-8674\(79\)90325-8](https://doi.org/10.1016/0092-8674(79)90325-8).
 31. Bak, A.L., Zeuthen, J., and Crick, F.H. (1977). Higher-order structure of human mitotic chromosomes. *Proc. Natl. Acad. Sci. USA* 74, 1595–1599. <https://doi.org/10.1073/pnas.74.4.1595>.
 32. Belmont, A.S., Sedat, J.W., and Agard, D.A. (1987). A three-dimensional approach to mitotic chromosome structure: evidence for a complex hierarchical organization. *J. Cell Biol.* 105, 77–92. <https://doi.org/10.1083/jcb.105.1.77>.
 33. Kireeva, N., Lakonishok, M., Kireev, I., Hirano, T., and Belmont, A.S. (2004). Visualization of early chromosome condensation: a hierarchical folding, axial glue model of chromosome structure. *J. Cell Biol.* 166, 775–785. <https://doi.org/10.1083/jcb.200406049>.
 34. Strukov, Y.G., Wang, Y., and Belmont, A.S. (2003). Engineered chromosome regions with altered sequence composition demonstrate hierarchical large-scale folding within metaphase chromosomes. *J. Cell Biol.* 162, 23–35. <https://doi.org/10.1083/jcb.200303098>.
 35. van den Engh, G., Sachs, R., and Trask, B.J. (1992). Estimating genomic distance from DNA sequence location in cell nuclei by a random walk model. *Science* 257, 1410–1412. <https://doi.org/10.1126/science.1388286>.
 36. Hahnfeldt, P., Hearst, J.E., Brenner, D.J., Sachs, R.K., and Hlatky, L.R. (1993). Polymer models for interphase chromosomes. *Proc. Natl. Acad. Sci. USA* 90, 7854–7858. <https://doi.org/10.1073/pnas.90.16.7854>.
 37. Yokota, H., van den Engh, G., Hearst, J.E., Sachs, R.K., and Trask, B.J. (1995). Evidence for the organization of chromatin in megabase pair-sized loops arranged along a random walk path in the human G0/G1 interphase nucleus. *J. Cell Biol.* 130, 1239–1249. <https://doi.org/10.1083/jcb.130.6.1239>.
 38. Sachs, R.K., van den Engh, G., Trask, B., Yokota, H., and Hearst, J.E. (1995). A random-walk/giant-loop model for interphase chromosomes. *Proc. Natl. Acad. Sci. USA* 92, 2710–2714. <https://doi.org/10.1073/pnas.92.7.2710>.
 39. Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J.D., Jacq, C., Johnston, M., et al. (1996). Life with 6000 genes. *Science* 274, 563–7. <https://doi.org/10.1126/science.274.5287.546>.
 40. C. Elegans Sequencing Consortium (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282, 2012–2018. <https://doi.org/10.1126/science.282.5396.2012>.
 41. Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., et al. (2000). The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185–2195. <https://doi.org/10.1126/science.287.5461.2185>.
 42. Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., et al. (2001). Initial sequencing and analysis of the human genome. *Nature* 409, 860–921. <https://doi.org/10.1038/35057062>.
 43. Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., et al. (2001). The sequence of the human genome. *Science* 291, 1304–1351. <https://doi.org/10.1126/science.1058040>.
 44. Mouse Genome Sequencing Consortium, Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P., Agarwala, R., Ainscough, R., Alexandersson, M., et al. (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature* 420, 520–562. <https://doi.org/10.1038/nature01262>.
 45. Christmas, M.J., Kaplow, I.M., Genereux, D.P., Dong, M.X., Hughes, G.M., Li, X., Sullivan, P.F., Hindle, A.G., Andrews, G., Armstrong, J.C., et al. (2023). Evolutionary constraint and innovation across hundreds of placental mammals. *Science* 380, eabn3943. <https://doi.org/10.1126/science.abn3943>.
 46. Stiller, J., Feng, S., Chowdhury, A.A., Rivas-González, I., Duchêne, D.A., Fang, Q., Deng, Y., Kozlov, A., Stamatakis, A., Claramunt, S., et al. (2024). Complexity of avian evolution revealed by family-level genomes. *Nature* 629, 851–860. <https://doi.org/10.1038/s41586-024-07323-1>.
 47. Wang, T., Antonacci-Fulton, L., Howe, K., Lawson, H.A., Lucas, J.K., Philippy, A.M., Popejoy, A.B., Asri, M., Carson, C., Chaisson, M.J.P., et al. (2022). The Human PanGenome Project: a global resource to map genomic diversity. *Nature* 604, 437–446. <https://doi.org/10.1038/s41586-022-04601-8>.
 48. Kaplan, N., and Dekker, J. (2013). High-throughput genome scaffolding from *in vivo* DNA interaction frequency. *Nat. Biotechnol.* 31, 1143–1147. <https://doi.org/10.1038/nbt.2768>.
 49. Burton, J.N., Adey, A., Patwardhan, R.P., Qiu, R., Kitzman, J.O., and Shendure, J. (2013). Chromosome-scale scaffolding of *de novo* genome assemblies based on chromatin interactions. *Nat. Biotechnol.* 31, 1119–1125. <https://doi.org/10.1038/nbt.2727>.
 50. Dudchenko, O., Batra, S.S., Omer, A.D., Nyquist, S.K., Hoeger, M., Durand, N.C., Shamim, M.S., Machol, I., Lander, E.S., Aiden, A.P., and Aiden, E.L. (2017). *De novo* assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science* 356, 92–95. <https://doi.org/10.1126/science.aal3327>.
 51. Hoencamp, C., Dudchenko, O., Elbatsh, A.M.O., Brahmachari, S., Raaijmakers, J.A., van Schaik, T., Sedeño Cacciatore, Á., Contessoto, V.G., van Heesbeen, R.G.H.P., van den Broek, B., et al. (2021). 3D genomics across the tree of life reveals condensin II as a determinant of architecture type. *Science* 372, 984–989. <https://doi.org/10.1126/science.abe2218>.
 52. Obinu, L., Trivedi, U., and Porceddu, A. (2023). Benchmarking of Hi-C tools for scaffolding *de novo* genome assemblies. Preprint at bioRxiv. <https://doi.org/10.1101/2023.05.16.540917>.
 53. Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002). Capturing Chromosome Conformation. *Science* 295, 1306–1311. <https://doi.org/10.1126/science.1067799>.
 54. Simonis, M., Klous, P., Splinter, E., Moshkin, Y., Willemse, R., de Wit, E., van Steensel, B., and de Laat, W. (2006). Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nat. Genet.* 38, 1348–1354. <https://doi.org/10.1038/ng1896>.
 55. Dostie, J., Richmond, T.A., Arnaout, R.A., Selzer, R.R., Lee, W.L., Honan, T.A., Rubio, E.D., Krumm, A., Lamb, J., Nusbaum, C., et al. (2006). Chromosome Conformation Capture Carbon Copy (5C): A Massively Parallel

- Solution for Mapping Interactions between Genomic Elements. *Genome Res.* 16, 1299–1309. <https://doi.org/10.1101/gr.557150>.
56. Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326, 289–293. <https://doi.org/10.1126/science.1181369>.
 57. Hsieh, T.H.S., Weiner, A., Lajoie, B.R., Dekker, J., Friedman, N., and Rando, O.J. (2015). Mapping nucleosome resolution chromosome folding in yeast by Micro-C. *Cell* 162, 108–119. <https://doi.org/10.1016/j.cell.2015.05.048>.
 58. Ma, W., Ay, F., Lee, C., Gulsoy, G., Deng, X., Cook, S., Hesson, J., Cavanaugh, C., Ware, C.B., Krumm, A., et al. (2015). Fine-scale chromatin interaction maps reveal the cis-regulatory landscape of human lincRNA genes. *Nat. Methods* 12, 71–78. <https://doi.org/10.1038/nmeth.3205>.
 59. Fullwood, M.J., Liu, M.H., Pan, Y.F., Liu, J., Xu, H., Mohamed, Y.B., Orlov, Y.L., Velkov, S., Ho, A., Mei, P.H., et al. (2009). An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* 462, 58–64. <https://doi.org/10.1038/nature08497>.
 60. Mumbach, M.R., Rubin, A.J., Flynn, R.A., Dai, C., Khavari, P.A., Greenleaf, W.J., and Chang, H.Y. (2016). HiChIP: efficient and sensitive analysis of protein-directed genome architecture. *Nat. Methods* 13, 919–922. <https://doi.org/10.1038/nmeth.3999>.
 61. Fang, R., Yu, M., Li, G., Chee, S., Liu, T., Schmitt, A.D., and Ren, B. (2016). Mapping of long-range chromatin interactions by proximity ligation-assisted ChIP-seq. *Cell Res.* 26, 1345–1348. <https://doi.org/10.1038/cr.2016.137>.
 62. van Steensel, B., and Henikoff, S. (2000). Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. *Nat. Biotechnol.* 18, 424–428. <https://doi.org/10.1038/74487>.
 63. Chen, Y., Zhang, Y., Wang, Y., Zhang, L., Brinkman, E.K., Adam, S.A., Goldman, R., van Steensel, B., Ma, J., and Belmont, A.S. (2018). Mapping 3D genome organization relative to nuclear compartments using TSA-Seq as a cytological ruler. *J. Cell Biol.* 217, 4025–4048. <https://doi.org/10.1083/jcb.201807108>.
 64. Beagrie, R.A., Scialdone, A., Schueler, M., Kraemer, D.C.A., Chotalia, M., Xie, S.Q., Barbieri, M., de Santiago, I., Lavitas, L.M., Branco, M.R., et al. (2017). Complex multi-enhancer contacts captured by genome architecture mapping. *Nature* 543, 519–524. <https://doi.org/10.1038/nature21411>.
 65. Quinodoz, S.A., Ollikainen, N., Tabak, B., Palla, A., Schmidt, J.M., Detmar, E., Lai, M.M., Shishkin, A.A., Bhat, P., Takei, Y., et al. (2018). Higher-Order Inter-chromosomal Hubs Shape 3D Genome Organization in the Nucleus. *Cell* 174, 744–757.e24. <https://doi.org/10.1016/j.cell.2018.05.024>.
 66. Nagano, T., Lubling, Y., Stevens, T.J., Schoenfelder, S., Yaffe, E., Dean, W., Laue, E.D., Tanay, A., and Fraser, P. (2013). Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature* 502, 59–64. <https://doi.org/10.1038/nature12593>.
 67. Nagano, T., Lubling, Y., Várnai, C., Dudley, C., Leung, W., Baran, Y., Mendelson Cohen, N., Wingett, S., Fraser, P., and Tanay, A. (2017). Cell-cycle dynamics of chromosomal organization at single-cell resolution. *Nature* 547, 61–67. <https://doi.org/10.1038/nature23001>.
 68. Ramani, V., Deng, X., Qiu, R., Gunderson, K.L., Steemers, F.J., Disteche, C.M., Noble, W.S., Duan, Z., and Shendure, J. (2017). Massively multiplex single-cell Hi-C. *Nat. Methods* 14, 263–266. <https://doi.org/10.1038/nmeth.4155>.
 69. Li, G., Liu, Y., Zhang, Y., Kubo, N., Yu, M., Fang, R., Kellis, M., and Ren, B. (2019). Joint profiling of DNA methylation and chromatin architecture in single cells. *Nat. Methods* 16, 991–993. <https://doi.org/10.1038/s41592-019-0502-z>.
 70. Kind, J., Pagie, L., de Vries, S.S., Nahidazar, L., Dey, S.S., Bienko, M., Zhan, Y., Lajoie, B., de Graaf, C.A., Amendola, M., et al. (2015). Genome-wide maps of nuclear lamina interactions in single human cells. *Cell* 163, 134–147. <https://doi.org/10.1016/j.cell.2015.08.040>.
 71. Tan, L., Xing, D., Chang, C.H., Li, H., and Xie, X.S. (2018). Three-dimensional genome structures of single diploid human cells. *Science* 361, 924–928. <https://doi.org/10.1126/science.aat5641>.
 72. Wang, S., Su, J.H., Beliveau, B.J., Bintu, B., Moffitt, J.R., Wu, C.T., and Zhuang, X. (2016). Spatial organization of chromatin domains and compartments in single chromosomes. *Science* 353, 598–602. <https://doi.org/10.1126/science.aaf8084>.
 73. Bintu, B., Mateo, L.J., Su, J.H., Sinnott-Armstrong, N.A., Parker, M., Kinrot, S., Yamaya, K., Boettiger, A.N., and Zhuang, X. (2018). Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science* 362, eaau1783. <https://doi.org/10.1126/science.aau1783>.
 74. Beliveau, B.J., Joyce, E.F., Apostolopoulos, N., Yilmaz, F., Fonseka, C.Y., McCole, R.B., Chang, Y., Li, J.B., Senaratne, T.N., Williams, B.R., et al. (2012). Versatile design and synthesis platform for visualizing genomes with Oligopaint FISH probes. *Proc. Natl. Acad. Sci. USA* 109, 21301–21306. <https://doi.org/10.1073/pnas.1213818110>.
 75. Nir, G., Farabella, I., Pérez Estrada, C., Ebeling, C.G., Beliveau, B.J., Sasaki, H.M., Lee, S.D., Nguyen, S.C., McCole, R.B., Chattoraj, S., et al. (2018). Walking along chromosomes with super-resolution imaging, contact maps, and integrative modeling. *PLoS Genet.* 14, e1007872. <https://doi.org/10.1371/journal.pgen.1007872>.
 76. Mateo, L.J., Murphy, S.E., Hafner, A., Cinquini, I.S., Walker, C.A., and Boettiger, A.N. (2019). Visualizing DNA folding and RNA in embryos at single-cell resolution. *Nature* 568, 49–54. <https://doi.org/10.1038/s41586-019-1035-4>.
 77. Mach, P., Kos, P.I., Zhan, Y., Cramard, J., Gaudin, S., Tünnermann, J., Marchi, E., Eglinger, J., Zuin, J., Kryzhanovska, M., et al. (2022). Cohesin and CTCF control the dynamics of chromosome folding. *Nat. Genet.* 54, 1907–1918. <https://doi.org/10.1038/s41588-022-01232-7>.
 78. Sikorav, J.L., and Jannink, G. (1994). Kinetics of chromosome condensation in the presence of topoisomerases: a phantom chain model. *Bioophys. J.* 66, 827–837. [https://doi.org/10.1016/s0006-3495\(94\)80859-8](https://doi.org/10.1016/s0006-3495(94)80859-8).
 79. Grosberg, A.Y., Nechaev, S.K., and Shakhnovich, E.I. (1988). The role of topological constraints in the kinetics of collapse of macromolecules. *J. Phys. France* 49, 2095–2100. <https://doi.org/10.1051/jphys:0198800490120209500>.
 80. Vologodskii, A.V., Levene, S.D., Klenin, K.V., Frank-Kamenetskii, M., and Cozzarelli, N.R. (1992). Conformational and thermodynamic properties of supercoiled DNA. *J. Mol. Biol.* 227, 1224–1243. [https://doi.org/10.1016/0022-2836\(92\)90533-p](https://doi.org/10.1016/0022-2836(92)90533-p).
 81. Goychuk, A., Kannan, D., Chakraborty, A.K., and Kardar, M. (2023). Polymer folding through active processes recreates features of genome organization. *Proc. Natl. Acad. Sci. USA* 120, e2221726120. <https://doi.org/10.1073/pnas.2221726120>.
 82. Chan, B., and Rubinstein, M. (2023). Theory of chromatin organization maintained by active loop extrusion. *Proc. Natl. Acad. Sci. USA* 120, e2222078120. <https://doi.org/10.1073/pnas.2222078120>.
 83. Chan, B., and Rubinstein, M. (2024). Activity-driven chromatin organization during interphase: Compaction, segregation, and entanglement suppression. *Proc. Natl. Acad. Sci. USA* 121, e2401494121. <https://doi.org/10.1073/pnas.2401494121>.
 84. Polovnikov, K.E., Slavov, B., Belan, S., Imakaev, M., Brandão, H.B., and Mirny, L.A. (2023). Crumpled polymer with loops recapitulates key features of chromosome organization. *Phys. Rev. X* 13, 041029. <https://doi.org/10.1103/physrevx.13.041029>.
 85. Keizer, V.I.P., Grosse-Holz, S., Woringer, M., Zambon, L., Aizel, K., Bongaerts, M., Delille, F., Kolar-Znika, L., Scolari, V.F., Hoffmann, S., et al. (2022). Live-cell micromanipulation of a genomic locus reveals interphase chromatin mechanics. *Science* 377, 489–495. <https://doi.org/10.1126/science.abi9810>.

86. Grosse-Holz, S. (2023). The searchable chromosome. *Trends Genet.* 39, 895–896. <https://doi.org/10.1016/j.tig.2023.08.006>.
87. Denker, A., and de Laat, W. (2016). The second decade of 3C technologies: detailed insights into nuclear organization. *Genes Dev.* 30, 1357–1382. <https://doi.org/10.1101/gad.281964.116>.
88. Goel, V.Y., and Hansen, A.S. (2021). The macro and micro of chromosome conformation capture. *Wiley Interdiscip. Rev. Dev. Biol.* 10, e395. <https://doi.org/10.1002/wdev.395>.
89. Jerkovic, I., and Cavalli, G. (2021). Understanding 3D genome organization by multidisciplinary methods. *Nat. Rev. Mol. Cell Biol.* 22, 511–528. <https://doi.org/10.1038/s41580-021-00362-w>.
90. Bouwman, B.A.M., Crosetto, N., and Bienko, M. (2022). The era of 3D and spatial genomics. *Trends Genet.* 38, 1062–1075. <https://doi.org/10.1016/j.tig.2022.05.010>.
91. Dekker, J., Alber, F., Aufmkolk, S., Beliveau, B.J., Bruneau, B.G., Belmont, A.S., Bintu, L., Boettiger, A., Calandrelli, R., Distefano, C.M., et al. (2023). Spatial and temporal organization of the genome: Current state and future aims of the 4D nucleome project. *Mol. Cell* 83, 2624–2640. <https://doi.org/10.1016/j.molcel.2023.06.018>.
92. Heitz, E. (1928). Das Heterochromatin der Moose. I. *Jahrb. Wiss. Bot.* 69, 762–818.
93. Croft, J.A., Bridger, J.M., Boyle, S., Perry, P., Teague, P., and Bickmore, W.A. (1999). Differences in the localization and morphology of chromosomes in the human nucleus. *J. Cell Biol.* 145, 1119–1131. <https://doi.org/10.1083/jcb.145.6.1119>.
94. Boyle, S., Gilchrist, S., Bridger, J.M., Mahy, N.L., Ellis, J.A., and Bickmore, W.A. (2001). The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. *Hum. Mol. Genet.* 10, 211–219. <https://doi.org/10.1093/hmg/10.3.211>.
95. Cremer, T., and Cremer, C. (2001). Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat. Rev. Genet.* 2, 292–301. <https://doi.org/10.1038/35066075>.
96. Dimitrova, D.S., and Gilbert, D.M. (1999). The spatial position and replication timing of chromosomal domains are both established in early G1 phase. *Mol. Cell* 4, 983–993. [https://doi.org/10.1016/s1097-2765\(00\)80227-0](https://doi.org/10.1016/s1097-2765(00)80227-0).
97. van Steensel, B., and Belmont, A.S. (2017). Lamina-Associated Domains: Links with Chromosome Architecture, Heterochromatin, and Gene Repression. *Cell* 169, 780–791. <https://doi.org/10.1016/j.cell.2017.04.022>.
98. Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de Klein, A., Wessels, L., de Laat, W., and van Steensel, B. (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* 453, 948–951. <https://doi.org/10.1038/nature06947>.
99. Harris, H.L., Gu, H., Olshansky, M., Wang, A., Farabella, I., Eliaz, Y., Kaliluchi, A., Krishna, A., Jacobs, M., Cauer, G., et al. (2023). Chromatin alternates between A and B compartments at kilobase scale for subgenomic organization. *Nat. Commun.* 14, 3303. <https://doi.org/10.1038/s41467-023-38429-1>.
100. Rao, S.S.P., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T., Sanborn, A.L., Machol, I., Omer, A.D., Lander, E.S., and Lieberman Aiden, E. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159, 1665–1680. <https://doi.org/10.1016/j.cell.2014.11.021>.
101. Spracklin, G., Abdennur, N., Imakaev, M., Chowdhury, N., Pradhan, S., Mirny, L.A., and Dekker, J. (2023). Diverse silent chromatin states modulate genome compartmentalization and loop extrusion barriers. *Nat. Struct. Mol. Biol.* 30, 38–51. <https://doi.org/10.1038/s41594-022-00892-7>.
102. Hildebrand, E.M., and Dekker, J. (2020). Mechanisms and Functions of Chromosome Compartmentalization. *Trends Biochem. Sci.* 45, 385–396. <https://doi.org/10.1016/j.tibs.2020.01.002>.
103. Haddad, N., Jost, D., and Vaillant, C. (2017). Perspectives: using polymer modeling to understand the formation and function of nuclear compartments. *Chromosome Res.* 25, 35–50. <https://doi.org/10.1007/s10577-016-9548-2>.
104. Falk, M., Feodorova, Y., Naumova, N., Imakaev, M., Lajoie, B.R., Leonhardt, H., Joffe, B., Dekker, J., Fudenberg, G., Solovei, I., and Mirny, L.A. (2019). Heterochromatin drives organization of conventional and inverted nuclei. *Nature* 570, 395–399.
105. Esposito, A., Bianco, S., Chiariello, A.M., Abraham, A., Fiorillo, L., Conte, M., Campanile, R., and Nicodemi, M. (2022). Polymer physics reveals a combinatorial code linking 3D chromatin architecture to 1D chromatin states. *Cell Rep.* 38, 110601. <https://doi.org/10.1016/j.celrep.2022.110601>.
106. Barbieri, M., Chotalia, M., Fraser, J., Lavitas, L.M., Dostie, J., Pombo, A., and Nicodemi, M. (2012). Complexity of chromatin folding is captured by the strings and binders switch model. *Proc. Natl. Acad. Sci. USA* 109, 16173–16178. <https://doi.org/10.1073/pnas.1204799109>.
107. Takemata, N., and Bell, S.D. (2021). Multi-scale architecture of archaeal chromosomes. *Mol. Cell* 81, 473–487.e6. <https://doi.org/10.1016/j.molcel.2020.12.001>.
108. Wang, Y., Zhang, Y., Zhang, R., van Schaik, T., Zhang, L., Sasaki, T., Peric-Hupkes, D., Chen, Y., Gilbert, D.M., van Steensel, B., et al. (2021). SPIN reveals genome-wide landscape of nuclear compartmentalization. *Genome Biol.* 22, 36. <https://doi.org/10.1186/s13059-020-02253-3>.
109. Gibson, B.A., Doolittle, L.K., Schneider, M.W.G., Jensen, L.E., Gamarra, N., Henry, L., Gerlich, D.W., Redding, S., and Rosen, M.K. (2019). Organization of Chromatin by Intrinsic and Regulated Phase Separation. *Cell* 179, 470–484.e21. <https://doi.org/10.1016/j.cell.2019.08.037>.
110. Strom, A.R., Emelyanov, A.V., Mir, M., Fyodorov, D.V., Darzacq, X., and Karpen, G.H. (2017). Phase separation drives heterochromatin domain formation. *Nature* 547, 241–245. <https://doi.org/10.1038/nature22989>.
111. Strom, A.R., Biggs, R.J., Banigan, E.J., Wang, X., Chiu, K., Herman, C., Collado, J., Yue, F., Ritland Politz, J.C., Tait, L.J., et al. (2021). HP1alpha is a chromatin crosslinker that controls nuclear and mitotic chromosome mechanics. *eLife* 10, e63972. <https://doi.org/10.7554/eLife.63972>.
112. Larson, A.G., Elnatan, D., Keenen, M.M., Trnka, M.J., Johnston, J.B., Burlingame, A.L., Agard, D.A., Redding, S., and Narlikar, G.J. (2017). Liquid droplet formation by HP1alpha suggests a role for phase separation in heterochromatin. *Nature* 547, 236–240. <https://doi.org/10.1038/nature22822>.
113. Sanulli, S., Trnka, M.J., Dharmarajan, V., Tibble, R.W., Pascal, B.D., Burlingame, A.L., Griffin, P.R., Gross, J.D., and Narlikar, G.J. (2019). HP1 reshapes nucleosome core to promote phase separation of heterochromatin. *Nature* 575, 390–394. <https://doi.org/10.1038/s41586-019-1669-2>.
114. Keenen, M.M., Brown, D., Brennan, L.D., Renger, R., Khoo, H., Carlson, C.R., Huang, B., Grill, S.W., Narlikar, G.J., and Redding, S. (2021). HP1 proteins compact DNA into mechanically and positionally stable phase separated domains. *eLife* 10, e64563. <https://doi.org/10.7554/eLife.64563>.
115. Zhao, H., Lin, Y., Lin, E., Liu, F., Shu, L., Jing, D., Wang, B., Wang, M., Shan, F., Zhang, L., et al. (2024). Genome folding principles uncovered in condensin-depleted mitotic chromosomes. *Nat. Genet.* 56, 1213–1224. <https://doi.org/10.1038/s41588-024-01759-x>.
116. Akilli, N., Cheutin, T., and Cavalli, G. (2024). Phase separation and inheritance of repressive chromatin domains. *Curr. Opin. Genet. Dev.* 36, 102201. <https://doi.org/10.1016/j.gde.2024.102201>.
117. Schuettengruber, B., Bourbon, H.M., Di Croce, L., and Cavalli, G. (2017). Genome Regulation by Polycomb and Trithorax: 70 Years and Counting. *Cell* 171, 34–57. <https://doi.org/10.1016/j.cell.2017.08.002>.
118. Girelli, G., Custodio, J., Kallas, T., Agostini, F., Wernersson, E., Spanjaard, B., Mota, A., Kolbeinsdottir, S., Gelali, E., Crosetto, N., and Bienko, M. (2024). The Role of Heterochromatin in Nuclear Architecture and Function. *Cell* 187, 6444–6464.e10. <https://doi.org/10.1016/j.cell.2024.09.010>.

- M. (2020). GPSeq reveals the radial organization of chromatin in the cell nucleus. *Nat. Biotechnol.* 38, 1184–1193. <https://doi.org/10.1038/s41587-020-0519-y>.
119. Bantignies, F., Roure, V., Comet, I., Leblanc, B., Schuettengruber, B., Bonnet, J., Tixier, V., Mas, A., and Cavalli, G. (2011). Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. *Cell* 144, 214–226. <https://doi.org/10.1016/j.cell.2010.12.026>.
120. Schoenfelder, S., Sugar, R., Dimond, A., Javierre, B.M., Armstrong, H., Mifsud, B., Dimitrova, E., Matheson, L., Tavares-Cadete, F., Furlan-Magaril, M., et al. (2015). Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. *Nat. Genet.* 47, 1179–1186. <https://doi.org/10.1038/ng.3393>.
121. Bonev, B., Mendelson Cohen, N., Szabo, Q., Fritsch, L., Papadopoulos, G.L., Lubling, Y., Xu, X., Lv, X., Hugnot, J.P., Tanay, A., and Cavalli, G. (2017). Multiscale 3D Genome Rewiring during Mouse Neural Development. *Cell* 171, 557–572.e24. <https://doi.org/10.1016/j.cell.2017.09.043>.
122. Jost, D., Carrivain, P., Cavalli, G., and Vaillant, C. (2014). Modeling epigenome folding: formation and dynamics of topologically associated chromatin domains. *Nucleic Acids Res.* 42, 9553–9561. <https://doi.org/10.1093/nar/gku698>.
123. Solovei, I., Kreysing, M., Lanctöt, C., Kösem, S., Peichl, L., Cremer, T., Guck, J., and Joffe, B. (2009). Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. *Cell* 137, 356–368. <https://doi.org/10.1016/j.cell.2009.01.052>.
124. Belaghzal, H., Borman, T., Stephens, A.D., Lafontaine, D.L., Venev, S.V., Weng, Z., Marko, J.F., and Dekker, J. (2021). Liquid chromatin Hi-C characterizes compartment-dependent chromatin interaction dynamics. *Nat. Genet.* 53, 367–378. <https://doi.org/10.1038/s41588-021-00784-4>.
125. Krietenstein, N., Abraham, S., Venev, S.V., Abdennur, N., Gibcus, J.H., Hsieh, T.S., Parsi, K.M., Yang, L., Maehr, R., Mirny, L.A., et al. (2020). Ultrastructural details of mammalian chromosome architecture. *Mol. Cell* 78, 554–565.e7. <https://doi.org/10.1016/j.molcel.2020.03.003>.
126. Hsieh, T.S., Cattoglio, C., Slobodyanyuk, E., Hansen, A.S., Darzacq, X., and Tjian, R. (2022). Enhancer-promoter interactions and transcription are largely maintained upon acute loss of CTCF, cohesin, WAPL or YY1. *Nat. Genet.* 54, 1919–1932. <https://doi.org/10.1038/s41588-022-01223-8>.
127. Friman, E.T., Flyamer, I.M., Marenduzzo, D., Boyle, S., and Bickmore, W.A. (2023). Ultra-long-range interactions between active regulatory elements. *Genome Res.* 33, 1269–1283. <https://doi.org/10.1101/gr.277567.122>.
128. Goel, V.Y., Huseyin, M.K., and Hansen, A.S. (2023). Region Capture Micro-C reveals coalescence of enhancers and promoters into nested microcompartments. *Nat. Genet.* 55, 1048–1056. <https://doi.org/10.1038/s41588-023-01391-1>.
129. Hua, P., Badat, M., Hanssen, L.L.P., Hentges, L.D., Crump, N., Downes, D.J., Jeziorska, D.M., Oudelaar, A.M., Schwessinger, R., Taylor, S., et al. (2021). Defining genome architecture at base-pair resolution. *Nature* 595, 125–129. <https://doi.org/10.1038/s41586-021-03639-4>.
130. Rosencrance, C.D., Ammour, H.N., Yu, Q., Ge, T., Rendleman, E.J., Marshall, S.A., and Eagen, K.P. (2020). Chromatin Hyperacetylation Impacts Chromosome Folding by Forming a Nuclear Subcompartment. *Mol. Cell* 78, 112–126.e12. <https://doi.org/10.1016/j.molcel.2020.03.018>.
131. Schooley, A., Venev, S.V., Aksanova, V., Navarrete, E., Dasso, M., and Dekker, J. (2024). Interphase chromosome conformation is specified by distinct folding programs inherited via mitotic chromosomes or through the cytoplasm. Preprint at bioRxiv. <https://doi.org/10.1101/2024.09.16.613305>.
132. Goel, V.Y., Aboreden, N.G., Jusuf, J.M., Zhang, H., Mori, L.P., Mirny, L.A., Blobel, G.A., Banigan, E.J., and Hansen, A.S. (2024). Dynamics of microcompartment formation at the mitosis-to-G1 transition. Preprint at bioRxiv. <https://doi.org/10.1101/2024.09.16.611917>.
133. Paulson, J.R., and Laemmli, U.K. (1977). The structure of histone-depleted metaphase chromosomes. *Cell* 12, 817–828. [https://doi.org/10.1016/0092-8674\(77\)90280-x](https://doi.org/10.1016/0092-8674(77)90280-x).
134. Earnshaw, W.C., and Laemmli, U.K. (1983). Architecture of metaphase chromosomes and chromosome scaffolds. *J. Cell Biol.* 96, 84–93. <https://doi.org/10.1083/jcb.96.1.84>.
135. Cook, P.R., and Brazell, I.A. (1975). Supercoils in human DNA. *J. Cell Sci.* 19, 261–279. <https://doi.org/10.1242/jcs.19.2.261>.
136. Nelson, W.G., Pienta, K.J., Barrack, E.R., and Coffey, D.S. (1986). The role of the nuclear matrix in the organization and function of DNA. *Annu. Rev. Biophys. Biophys. Chem.* 15, 457–475. <https://doi.org/10.1146/annurev.bb.15.060186.002325>.
137. Ostashevsky, J. (1998). A polymer model for the structural organization of chromatin loops and minibands in interphase chromosomes. *Mol. Biol. Cell* 9, 3031–3040. <https://doi.org/10.1091/mbc.9.11.3031>.
138. Wood, C., and Tonegawa, S. (1983). Diversity and joining segments of mouse immunoglobulin heavy chain genes are closely linked and in the same orientation: implications for the joining mechanism. *Proc. Natl. Acad. Sci. USA* 80, 3030–3034. <https://doi.org/10.1073/pnas.80.10.3030>.
139. Riggs, A.D. (1990). 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.* 326, 285–297. <https://doi.org/10.1098/rstb.1990.0012>.
140. Blackwood, E.M., and Kadonaga, J.T. (1998). Going the Distance: A Current View of Enhancer Action. *Science* 281, 60–63. <https://doi.org/10.1126/science.281.5373.60>.
141. Kimura, K., Rybenkov, V.V., Crisponi, N.J., Hirano, T., and Cozzarelli, N.R. (1999). 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. *Cell* 98, 239–248. [https://doi.org/10.1016/s0092-8674\(00\)81018-1](https://doi.org/10.1016/s0092-8674(00)81018-1).
142. Nasmyth, K. (2001). Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* 35, 673–745. <https://doi.org/10.1146/annurev.genet.35.102401.091334>.
143. Kagey, M.H., Newman, J.J., Bilodeau, S., Zhan, Y., Orlando, D.A., van Berkum, N.L., Ebmeier, C.C., Goossens, J., Rahl, P.B., Levine, S.S., et al. (2010). Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467, 430–435. <https://doi.org/10.1038/nature09380>.
144. Merkenschlager, M. (2010). Cohesin: a global player in chromosome biology with local ties to gene regulation. *Curr. Opin. Genet. Dev.* 20, 555–561. <https://doi.org/10.1016/j.gde.2010.05.007>.
145. Hirano, T. (2002). The ABCs of SMC proteins: two-armed ATPases for chromosome condensation, cohesion, and repair. *Genes Dev.* 16, 399–414. <https://doi.org/10.1101/gad.955102>.
146. Strick, T.R., Kawaguchi, T., and Hirano, T. (2004). Real-time detection of single-molecule DNA compaction by condensin I. *Curr. Biol.* 14, 874–880. <https://doi.org/10.1016/j.cub.2004.04.038>.
147. Alipour, E., and Marko, J.F. (2012). Self-organization of domain structures by DNA-loop-extruding enzymes. *Nucleic Acids Res.* 40, 11202–11212. <https://doi.org/10.1093/nar/gks925>.
148. Goloborodko, A., Imakaev, M.V., Marko, J.F., and Mirny, L. (2016). Compaction and segregation of sister chromatids via active loop extrusion. *eLife* 5, e14864. <https://doi.org/10.7554/eLife.14864>.
149. Fudenberg, G., Imakaev, M., Lu, C., Goloborodko, A., Abdennur, N., and Mirny, L.A. (2016). Formation of chromosomal domains by loop extrusion. *Cell Rep.* 15, 2038–2049. <https://doi.org/10.1016/j.celrep.2016.04.085>.
150. Sanborn, A.L., Rao, S.S.P., Huang, S.C., Durand, N.C., Huntley, M.H., Jewett, A.I., Bochkov, I.D., Chinnappan, D., Cutkosky, A., Li, J., et al. (2015). Chromatin extrusion explains key features of loop and domain

- formation in wild-type and engineered genomes. Proc. Natl. Acad. Sci. USA 112, E6456–E6465. <https://doi.org/10.1073/pnas.1518552112>.
151. Nora, E.P., Lajoie, B.R., Schulz, E.G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., van Berkum, N.L., Meisig, J., Sedat, J., et al. (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485, 381–385. <https://doi.org/10.1038/nature11049>.
 152. Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380. <https://doi.org/10.1038/nature11082>.
 153. Nora, E.P., Goloborodko, A., Valton, A.L., Gibcus, J.H., Uebersohn, A., Abdennur, N., Dekker, J., Mirny, L.A., and Bruneau, B.G. (2017). Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. Cell 169, 930–944.e22. <https://doi.org/10.1016/j.cell.2017.05.004>.
 154. Rao, S.S.P., Huang, S.C., Glenn St Hilaire, B., Engreitz, J.M., Perez, E.M., Kieffer-Kwon, K.R., Sanborn, A.L., Johnstone, S.E., Bascom, G.D., et al. (2017). Cohesin Loss Eliminates All Loop Domains. Cell 171, 305–320.e24. <https://doi.org/10.1016/j.cell.2017.09.026>.
 155. Fudenberg, G., Abdennur, N., Imakaev, M., Goloborodko, A., and Mirny, L.A. (2017). Emerging Evidence of Chromosome Folding by Loop Extrusion. Cold Spring Harb. Symp. Quant. Biol. 82, 45–55. <https://doi.org/10.1101/sqb.2017.82.034710>.
 156. Davidson, I.F., and Peters, J.M. (2021). Genome folding through loop extrusion by SMC complexes. Nat. Rev. Mol. Cell Biol. 22, 445–464. <https://doi.org/10.1038/s41580-021-00349-7>.
 157. Hoencamp, C., and Rowland, B.D. (2023). Genome control by SMC complexes. Nat. Rev. Mol. Cell Biol. 24, 633–650. <https://doi.org/10.1038/s41580-023-00609-8>.
 158. Goloborodko, A., Marko, J.F., and Mirny, L.A. (2016). Chromosome Compaction by Active Loop Extrusion. Biophys. J. 110, 2162–2168. <https://doi.org/10.1016/j.bpj.2016.02.041>.
 159. Hirano, T., Kobayashi, R., and Hirano, M. (1997). Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a Xenopus homolog of the Drosophila Barren protein. Cell 89, 511–521. [https://doi.org/10.1016/s0092-8674\(00\)80233-0](https://doi.org/10.1016/s0092-8674(00)80233-0).
 160. Hirano, T., and Mitchison, T.J. (1994). A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell 79, 449–458. [https://doi.org/10.1016/0092-8674\(94\)90254-2](https://doi.org/10.1016/0092-8674(94)90254-2).
 161. Saitoh, N., Goldberg, I.G., Wood, E.R., and Earnshaw, W.C. (1994). Scll: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. J. Cell Biol. 127, 303–318. <https://doi.org/10.1083/jcb.127.2.303>.
 162. Ganji, M., Shaltiel, I.A., Bisht, S., Kim, E., Kalichava, A., Haering, C.H., and Dekker, C. (2018). Real-time imaging of DNA loop extrusion by condensin. Science 360, 102–105. <https://doi.org/10.1126/science.aar7831>.
 163. Davidson, I.F., Bauer, B., Goetz, D., Tang, W., Wutz, G., and Peters, J.M. (2019). DNA loop extrusion by human cohesin. Science 366, 1338–1345. <https://doi.org/10.1126/science.aaz3418>.
 164. Golfier, S., Quail, T., Kimura, H., and Brugués, J. (2020). Cohesin and condensin extrude DNA loops in a cell cycle-dependent manner. eLife 9. <https://doi.org/10.7554/elife.53885>.
 165. Kong, M., Cutts, E.E., Pan, D., Beuron, F., Kaliyappan, T., Xue, C., Morris, E.P., Musacchio, A., Vannini, A., and Greene, E.C. (2020). Human Condensin I and II Drive Extensive ATP-Dependent Compaction of Nucleosome-Bound DNA. Mol. Cell 79, 99–114.e9. <https://doi.org/10.1016/j.molcel.2020.04.026>.
 166. Jeppsson, K., Pradhan, B., Sutani, T., Sakata, T., Umeda Igarashi, M., Berta, D.G., Kanno, T., Nakato, R., Shirahige, K., Kim, E., and Björkergren, C. (2024). Loop-extruding Smc5/6 organizes transcription-induced positive DNA supercoils. Mol. Cell 84, 867–882.e5. <https://doi.org/10.1016/j.molcel.2024.01.005>.
 167. Barth, R., Davidson, I.F., van der Torre, J., Taschner, M., Gruber, S., Peters, J.-M., and Dekker, C. (2023). SMC motor proteins extrude DNA asymmetrically and contain a direction switch. Preprint at bioRxiv. <https://doi.org/10.1101/2023.12.21.572892>.
 168. Banigan, E.J., Tang, W., van den Berg, A.A., Stocsits, R.R., Wutz, G., Brandão, H.B., Busslinger, G.A., Peters, J.M., and Mirny, L.A. (2023). Transcription shapes 3D chromatin organization by interacting with loop extrusion. Proc. Natl. Acad. Sci. USA 120, e2210480120. <https://doi.org/10.1073/pnas.2210480120>.
 169. Li, Y., Haarhuis, J.H.I., Sedeño Cacciatore, Á., Oldenkamp, R., van Ruiten, M.S., Willems, L., Teunissen, H., Muir, K.W., de Wit, E., Rowland, B.D., and Panne, D. (2020). The structural basis for cohesin-CTCF-anchored loops. Nature 578, 472–476. <https://doi.org/10.1038/s41586-019-1910-z>.
 170. Davidson, I.F., Barth, R., Zaczek, M., van der Torre, J., Tang, W., Nagasaka, K., Janissen, R., Kerssemakers, J., Wutz, G., Dekker, C., and Peters, J.M. (2023). CTCF is a DNA-tension-dependent barrier to cohesin-mediated loop extrusion. Nature 616, 822–827. <https://doi.org/10.1038/s41586-023-05961-5>.
 171. Kim, E., Kerssemakers, J., Shaltiel, I.A., Haering, C.H., and Dekker, C. (2020). DNA-loop extruding condensin complexes can traverse one another. Nature 579, 438–442. <https://doi.org/10.1038/s41586-020-2067-5>.
 172. Pradhan, B., Barth, R., Kim, E., Davidson, I.F., Bauer, B., van Laar, T., Yang, W., Ryu, J.K., van der Torre, J., Peters, J.M., and Dekker, C. (2022). SMC complexes can traverse physical roadblocks bigger than their ring size. Cell Rep. 41, 111491. <https://doi.org/10.1016/j.celrep.2022.111491>.
 173. Dekker, C., Haering, C.H., Peters, J.M., and Rowland, B.D. (2023). How do molecular motors fold the genome? Science 382, 646–648. <https://doi.org/10.1126/science.adl8308>.
 174. Nomidis, S.K., Carlon, E., Gruber, S., and Marko, J.F. (2022). DNA tension-modulated translocation and loop extrusion by SMC complexes revealed by molecular dynamics simulations. Nucleic Acids Res. 50, 4974–4987. <https://doi.org/10.1093/nar/gkac268>.
 175. Wang, X., Le, T.B.K., Lajoie, B.R., Dekker, J., Laub, M.T., and Rudner, D.Z. (2015). Condensin promotes the juxtaposition of DNA flanking its loading site in *Bacillus subtilis*. Genes Dev. 29, 1661–1675. <https://doi.org/10.1101/gad.265876.115>.
 176. Le, T.B.K., Imakaev, M.V., Mirny, L.A., and Laub, M.T. (2013). High-resolution mapping of the spatial organization of a bacterial chromosome. Science 342, 731–734. <https://doi.org/10.1126/science.1242059>.
 177. Umbarger, M.A., Toro, E., Wright, M.A., Porreca, G.J., Baù, D., Hong, S.H., Fero, M.J., Zhu, L.J., Martí-Renom, M.A., McAdams, H.H., et al. (2011). The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation. Mol. Cell 44, 252–264. <https://doi.org/10.1016/j.molcel.2011.09.010>.
 178. Sullivan, N.L., Marquis, K.A., and Rudner, D.Z. (2009). Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. Cell 137, 697–707. <https://doi.org/10.1016/j.cell.2009.04.044>.
 179. Wang, X., Hughes, A.C., Brandão, H.B., Walker, B., Lierz, C., Cochran, J.C., Oakley, M.G., Kruse, A.C., and Rudner, D.Z. (2018). In Vivo Evidence for ATPase-Dependent DNA Translocation by the *Bacillus subtilis* SMC Condensin Complex. Mol. Cell 71, 841–847.e5. <https://doi.org/10.1016/j.molcel.2018.07.006>.
 180. Dequeker, B.J.H., Scherr, M.J., Brandão, H.B., Gassler, J., Powell, S., Gaspar, I., Flyamer, I.M., Lalic, A., Tang, W., Stocsits, R., et al. (2022). MCM complexes are barriers that restrict cohesin-mediated loop extrusion. Nature 606, 197–203. <https://doi.org/10.1038/s41586-022-04730-0>.
 181. Chang, L.H., and Noordermeer, D. (2024). Permeable TAD boundaries and their impact on genome-associated functions. BioEssays 46, e2400137. <https://doi.org/10.1002/bies.202400137>.

182. Liu, N.Q., Maresca, M., van den Brand, T., Braccioli, L., Schijns, M.M.G.A., Teunissen, H., Bruneau, B.G., Nora, E.P., and de Wit, E. (2021). WAPL maintains a cohesin loading cycle to preserve cell-type-specific distal gene regulation. *Nat. Genet.* 53, 100–109. <https://doi.org/10.1038/s41588-020-00744-4>.
183. Valton, A.L., Venev, S.V., Mair, B., Khokhar, E.S., Tong, A.H.Y., Usaj, M., Chan, K., Pai, A.A., Moffat, J., and Dekker, J. (2022). A cohesin traffic pattern genetically linked to gene regulation. *Nat. Struct. Mol. Biol.* 29, 1239–1251. <https://doi.org/10.1038/s41594-022-00890-9>.
184. Bastié, N., Chapard, C., Cournac, A., Nejmi, S., Mboumba, H., Gadal, O., Thierry, A., Beckouët, F., and Koszul, R. (2024). Sister chromatid cohesion halts DNA loop expansion. *Mol. Cell* 84, 1139–1148.e5. <https://doi.org/10.1016/j.molcel.2024.02.004>.
185. Brandão, H.B., Ren, Z., Karabojá, X., Mirny, L.A., and Wang, X. (2021). DNA-loop-extruding SMC complexes can traverse one another in vivo. *Nat. Struct. Mol. Biol.* 28, 642–651. <https://doi.org/10.1038/s41594-021-00626-1>.
186. Rinzenma, N.J., Sofiadis, K., Tjalsma, S.J.D., Verstegen, M.J.A.M., Oz, Y., Valdes-Quetzada, C., Felder, A.K., Filipovska, T., van der Elst, S., de Andrade Dos Ramos, Z., et al. (2022). Building regulatory landscapes reveals that an enhancer can recruit cohesin to create contact domains, engage CTCF sites and activate distant genes. *Nat. Struct. Mol. Biol.* 29, 563–574. <https://doi.org/10.1038/s41594-022-00787-7>.
187. Gruber, S., and Errington, J. (2009). Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. *Cell* 137, 685–696. <https://doi.org/10.1016/j.cell.2009.02.035>.
188. Galitsyna, A., Ulianov, S.V., Bykov, N.S., Veil, M., Gao, M., Perevoschikova, K., Gelfand, M., Razin, S.V., Mirny, L., and Onichtchouk, D. (2023). Extrusion fountains are hallmarks of chromosome organization emerging upon zygotic genome activation. Preprint at bioRxiv. <https://doi.org/10.1101/2023.07.15.549120>.
189. Gil, J., Rosin, L.F., Navarrete, E., Chowdhury, N., Abraham, S., Cornilleau, G., Lei, E.P., Mozziconacci, J., Mirny, L.A., Muller, H., and Drinnenberg, I.A. (2024). Unique territorial and compartmental organization of chromosomes in the holocentric silkworm. Preprint at bioRxiv. <https://doi.org/10.1101/2023.09.14.557757>.
190. Busslinger, G.A., Stocsits, R.R., van der Lelij, P., Axelsson, E., Tedeschi, A., Gajjar, N., and Peters, J.M. (2017). Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl. *Nature* 544, 503–507. <https://doi.org/10.1038/nature22063>.
191. Natale, F., Rapp, A., Yu, W., Maiser, A., Harz, H., Scholl, A., Grulich, S., Anton, T., Hörl, D., Chen, W., et al. (2017). Identification of the elementary structural units of the DNA damage response. *Nat. Commun.* 8, 15760. <https://doi.org/10.1038/ncomms15760>.
192. Arnould, C., Rocher, V., Finoux, A.L., Clouaire, T., Li, K., Zhou, F., Caron, P., Mangeot, P.E., Ricci, E.P., Mourad, R., et al. (2021). Loop extrusion as a mechanism for formation of DNA damage repair foci. *Nature* 590, 660–665. <https://doi.org/10.1038/s41586-021-03193-z>.
193. Amendola, M., and van Steensel, B. (2015). Nuclear lamins are not required for lamina-associated domain organization in mouse embryonic stem cells. *EMBO Rep.* 16, 610–617. <https://doi.org/10.15252/embr.201439789>.
194. Manzo, S.G., Dauban, L., and van Steensel, B. (2022). Lamina-associated domains: tethers and looseners. *Curr. Opin. Cell Biol.* 74, 80–87. <https://doi.org/10.1016/j.ceb.2022.01.004>.
195. Solovei, I., Wang, A.S., Thanisch, K., Schmidt, C.S., Krebs, S., Zwerger, M., Cohen, T.V., Devys, D., Foisner, R., Peichl, L., et al. (2013). LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell* 152, 584–598. <https://doi.org/10.1016/j.cell.2013.01.009>.
196. Yu, R., Roseman, S., Siegenfeld, A.P., Nguyen, S.C., Joyce, E.F., Liu, B.B., Krantz, I.D., Alexander, K.A., and Berger, S.L. (2023). CTCF/cohesin organize the ground state of chromatin-nuclear speckle association. Preprint at bioRxiv. <https://doi.org/10.1101/2023.07.22.550178>.
197. Blobel, G. (1985). Gene gating: a hypothesis. *Proc. Natl. Acad. Sci. USA* 82, 8527–8529. <https://doi.org/10.1073/pnas.82.24.8527>.
198. Toro, E., Hong, S.H., McAdams, H.H., and Shapiro, L. (2008). Caulobacter requires a dedicated mechanism to initiate chromosome segregation. *Proc. Natl. Acad. Sci. USA* 105, 15435–15440. <https://doi.org/10.1073/pnas.0807448105>.
199. Treilles-Sticken, E., Loidl, J., and Scherthan, H. (1999). Bouquet formation in budding yeast: initiation of recombination is not required for meiotic telomere clustering. *J. Cell Sci.* 112, 651–658. <https://doi.org/10.1242/jcs.112.5.651>.
200. Wasserman, S.A., and Cozzarelli, N.R. (1986). Biochemical topology: applications to DNA recombination and replication. *Science* 232, 951–960. <https://doi.org/10.1126/science.3010458>.
201. Wood, E.R., and Earnshaw, W.C. (1990). Mitotic chromatin condensation in vitro using somatic cell extracts and nuclei with variable levels of endogenous topoisomerase II. *J. Cell Biol.* 111, 2839–2850. <https://doi.org/10.1083/jcb.111.6.2839>.
202. de Gennes, P.-G. (1979). *Scaling Theory of Polymer Physics* (Cornell University Press).
203. Grosberg, A.Y., Rabin, Y., Havlin, S., and Neer, A. (1993). Crumpled globule model of the three-dimensional structure of DNA. *Europhys. Lett.* 23, 373–378. <https://doi.org/10.1209/0295-5075/23/5/012>.
204. Rosa, A., and Everaers, R. (2008). Structure and dynamics of interphase chromosomes. *PLoS Comput. Biol.* 4, e1000153. <https://doi.org/10.1371/journal.pcbi.1000153>.
205. Boettiger, A.N., Bintu, B., Moffitt, J.R., Wang, S., Beliveau, B.J., Fudenberg, G., Imakaev, M., Mirny, L.A., Wu, C.T., and Zhuang, X. (2016). Super-resolution imaging reveals distinct chromatin folding for different epigenetic states. *Nature* 529, 418–422. <https://doi.org/10.1038/nature16496>.
206. Halverson, J.D., Smrek, J., Kremer, K., and Grosberg, A.Y. (2014). From a melt of rings to chromosome territories: the role of topological constraints in genome folding. *Rep. Prog. Phys.* 77, 022601. <https://doi.org/10.1088/0034-4885/77/2/022601>.
207. Tavares-Cadete, F., Norouzi, D., Dekker, B., Liu, Y., and Dekker, J. (2020). Multi-contact 3C data reveal that the human genome is largely unentangled. Preprint at bioRxiv. <https://doi.org/10.1101/2020.03.03.975425>.
208. Hildebrand, E.M., Polovnikov, K., Dekker, B., Liu, Y., Lafontaine, D.L., Fox, A.N., Li, Y., Venev, S.V., Mirny, L.A., and Dekker, J. (2024). Mitotic chromosomes are self-entangled and disentangle through a topoisomerase-II-dependent two-stage exit from mitosis. *Mol. Cell* 84, 1422–1441.e14. <https://doi.org/10.1016/j.molcel.2024.02.025>.
209. Orlandini, E., Marenduzzo, D., and Micheletto, D. (2019). Synergy of topoisomerase and structural-maintenance-of-chromosomes proteins creates a universal pathway to simplify genome topology. *Proc. Natl. Acad. Sci. USA* 116, 8149–8154. <https://doi.org/10.1073/pnas.1815394116>.
210. Hirano, T., and Mitchison, T.J. (1991). Cell cycle control of higher-order chromatin assembly around naked DNA in vitro. *J. Cell Biol.* 115, 1479–1489. <https://doi.org/10.1083/jcb.115.6.1479>.
211. Shintomi, K., and Hirano, T. (2021). Guiding functions of the C-terminal domain of topoisomerase IIalpha advance mitotic chromosome assembly. *Nat. Commun.* 12, 2917. <https://doi.org/10.1038/s41467-021-23205-w>.
212. Kawamura, R., Pope, L.H., Christensen, M.O., Sun, M., Terekhova, K., Boege, F., Mielke, C., Andersen, A.H., and Marko, J.F. (2010). Mitotic chromosomes are constrained by topoisomerase II-sensitive DNA entanglements. *J. Cell Biol.* 188, 653–663. <https://doi.org/10.1083/jcb.200910085>.
213. Su, J.H., Zheng, P., Kinrot, S.S., Bintu, B., and Zhuang, X. (2020). Genome-Scale Imaging of the 3D Organization and Transcriptional

- Activity of Chromatin. *Cell* 182, 1641–1659.e26. <https://doi.org/10.1016/j.cell.2020.07.032>.
214. Schwarzer, W., Abdennur, N., Goloborodko, A., Pekowska, A., Fudenberg, G., Loe-Mie, Y., Fonseca, N.A., Huber, W., Haering, C.H., Mirny, L., et al. (2017). Two independent modes of chromatin organization revealed by cohesin removal. *Nature* 551, 51–56. <https://doi.org/10.1038/nature24281>.
215. Tjong, H., Gong, K., Chen, L., and Alber, F. (2012). Physical tethering and volume exclusion determine higher-order genome organization in budding yeast. *Genome Res.* 22, 1295–1305. <https://doi.org/10.1101/gr.129437.111>.
216. Schalbetter, S.A., Goloborodko, A., Fudenberg, G., Belton, J.M., Miles, C., Yu, M., Dekker, J., Mirny, L., and Baxter, J. (2017). SMC complexes differentially compact mitotic chromosomes according to genomic context. *Nat. Cell Biol.* 19, 1071–1080. <https://doi.org/10.1038/ncb3594>.
217. Wong, H., Marie-Nelly, H., Herbert, S., Carrivain, P., Blanc, H., Koszul, R., Fabre, E., and Zimmer, C. (2012). A predictive computational model of the dynamic 3D interphase yeast nucleus. *Curr. Biol.* 22, 1881–1890. <https://doi.org/10.1016/j.cub.2012.07.069>.
218. Tortora, M.M., Salari, H., and Jost, D. (2020). Chromosome dynamics during interphase: a biophysical perspective. *Curr. Opin. Genet. Dev.* 61, 37–43. <https://doi.org/10.1016/j.gde.2020.03.001>.
219. Strickfaden, H., Zunhammer, A., van Koningsbruggen, S., Köhler, D., and Cremer, T. (2010). 4D chromatin dynamics in cycling cells: Theodor Boveri's hypotheses revisited. *Nucleus* 1, 284–297. <https://doi.org/10.4161/nucl.1.3.11969>.
220. Weber, S.C., Spakowitz, A.J., and Theriot, J.A. (2010). Bacterial chromosomal loci move subdiffusively through a viscoelastic cytoplasm. *Phys. Rev. Lett.* 104, 238102. <https://doi.org/10.1103/PhysRevLett.104.238102>.
221. Hajjoul, H., Mathon, J., Ranchon, H., Goiffon, I., Mozziconacci, J., Albert, B., Carrivain, P., Victor, J.M., Gadal, O., Bystricky, K., and Bancaud, A. (2013). High-throughput chromatin motion tracking in living yeast reveals the flexibility of the fiber throughout the genome. *Genome Res.* 23, 1829–1838. <https://doi.org/10.1101/gr.157008.113>.
222. Khanna, N., Zhang, Y., Lucas, J.S., Dudko, O.K., and Murre, C. (2019). Chromosome dynamics near the sol-gel phase transition dictate the timing of remote genomic interactions. *Nat. Commun.* 10, 2771. <https://doi.org/10.1038/s41467-019-10628-9>.
223. Lucas, J.S., Zhang, Y., Dudko, O.K., and Murre, C. (2014). 3D trajectories adopted by coding and regulatory DNA elements: first-passage times for genomic interactions. *Cell* 158, 339–352. <https://doi.org/10.1016/j.cell.2014.05.036>.
224. Miné-Hattab, J., Recamier, V., Izeddin, I., Rothstein, R., and Darzacq, X. (2017). Multi-scale tracking reveals scale-dependent chromatin dynamics after DNA damage. *Mol. Biol. Cell* 28, 3323–3332. <https://doi.org/10.1091/mbc.E17-05-0317>.
225. Bronshtein, I., Kepten, E., Kanter, I., Berezin, S., Lindner, M., Redwood, A.B., Mai, S., Gonzalo, S., Foisner, R., Shav-Tal, Y., and Garini, Y. (2015). Loss of lamin A function increases chromatin dynamics in the nuclear interior. *Nat. Commun.* 6, 8044. <https://doi.org/10.1038/ncomms9044>.
226. Tamm, M.V., and Polovnikov, K. (2018). Dynamics of Polymers: Classic Results and Recent Developments. Chapter 3. In *Order, Disorder and Criticality—Advanced Problems of Phase Transition Theory*, 5, Holovatch., ed. (National Academy of Sciences), pp. 113–172.
227. Brückner, D.B., Chen, H., Barinov, L., Zoller, B., and Gregor, T. (2023). Stochastic motion and transcriptional dynamics of pairs of distal DNA loci on a compacted chromosome. *Science* 380, 1357–1362. <https://doi.org/10.1126/science.adf5568>.
228. Kleckner, N., Zickler, D., Jones, G.H., Dekker, J., Padmore, R., Henle, J., and Hutchinson, J. (2004). A mechanical basis for chromosome function. *Proc. Natl. Acad. Sci. USA* 101, 12592–12597. <https://doi.org/10.1073/pnas.0402724101>.
229. Marko, J.F., and Siggia, E.D. (1997). Polymer models of meiotic and mitotic chromosomes. *Mol. Biol. Cell* 8, 2217–2231. <https://doi.org/10.1091/mcb.8.11.2217>.
230. Marko, J.F. (2008). Micromechanical studies of mitotic chromosomes. *Chromosome Res.* 16, 469–497. <https://doi.org/10.1007/s10577-008-1233-7>.
231. Stephens, A.D., Banigan, E.J., Adam, S.A., Goldman, R.D., and Marko, J.F. (2017). Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus. *Mol. Biol. Cell* 28, 1984–1996. <https://doi.org/10.1091/mbc.E16-09-0653>.
232. Stephens, A.D., Liu, P.Z., Banigan, E.J., Almassalha, L.M., Backman, V., Adam, S.A., Goldman, R.D., and Marko, J.F. (2018). Chromatin histone modifications and rigidity affect nuclear morphology independent of lamins. *Mol. Biol. Cell* 29, 220–233. <https://doi.org/10.1091/mbc.E17-06-0410>.
233. Poirier, M.G., and Marko, J.F. (2002). Micromechanical studies of mitotic chromosomes. *J. Muscle Res Cell* 23, 409–431.
234. Biggs, R., Liu, P.Z., Stephens, A.D., and Marko, J.F. (2019). Effects of altering histone posttranslational modifications on mitotic chromosome structure and mechanics. *Mol. Biol. Cell* 30, 820–827. <https://doi.org/10.1091/mbc.E18-09-0592>.
235. Rowley, M.J., Nichols, M.H., Lyu, X., Ando-Kuri, M., Rivera, I.S.M., Hermetz, K., Wang, P., Ruan, Y., and Corces, V.G. (2017). Evolutionarily Conserved Principles Predict 3D Chromatin Organization. *Mol. Cell* 67, 837–852.e7. <https://doi.org/10.1016/j.molcel.2017.07.022>.
236. Mirny, L., and Dekker, J. (2022). Mechanisms of Chromosome Folding and Nuclear Organization: Their Interplay and Open Questions. *Cold Spring Harb. Perspect. Biol.* 14, a040147. <https://doi.org/10.1101/cshperspect.a040147>.
237. Nuebler, J., Fudenberg, G., Imakaev, M., Abdennur, N., and Mirny, L.A. (2018). Chromatin organization by an interplay of loop extrusion and compartmental segregation. *Proc. Natl. Acad. Sci. USA* 115, E6697–E6706. <https://doi.org/10.1073/pnas.1717730115>.
238. Haarhuis, J.H.I., van der Weide, R.H., Blomen, V.A., Yáñez-Cuna, J.O., Amendola, M., van Ruiten, M.S., Krijger, P.H.L., Teunissen, H., Medema, R.H., van Steensel, B., et al. (2017). The Cohesin Release Factor WAPL Restricts Chromatin Loop Extension. *Cell* 169, 693–707.e14. <https://doi.org/10.1016/j.cell.2017.04.013>.
239. Tan, L., Xing, D., Daley, N., and Xie, X.S. (2019). Three-dimensional genome structures of single sensory neurons in mouse visual and olfactory systems. *Nat. Struct. Mol. Biol.* 26, 297–307. <https://doi.org/10.1038/s41594-019-0205-2>.
240. Ono, T., Losada, A., Hirano, M., Myers, M.P., Neuwald, A.F., and Hirano, T. (2003). Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. *Cell* 115, 109–121. [https://doi.org/10.1016/s0092-8674\(03\)00724-4](https://doi.org/10.1016/s0092-8674(03)00724-4).
241. Chu, L., Liang, Z., Mukhina, M., Fisher, J., Vincenten, N., Zhang, Z., Hutchinson, J., Zickler, D., and Kleckner, N. (2020). The 3D Topography of Mitotic Chromosomes. *Mol. Cell* 79, 902–916.e6. <https://doi.org/10.1016/j.molcel.2020.07.002>.
242. Schneider, M.W.G., Gibson, B.A., Otsuka, S., Spicer, M.F.D., Petrovic, M., Blaukopf, C., Langer, C.C.H., Batty, P., Nagaraju, T., Doolittle, L.K., et al. (2022). A mitotic chromatin phase transition prevents perforation by microtubules. *Nature* 609, 183–190. <https://doi.org/10.1038/s41586-022-05027-y>.
243. Gerlich, D., Koch, B., Dupeux, F., Peters, J.M., and Ellenberg, J. (2006). Live-cell imaging reveals a stable cohesin-chromatin interaction after but not before DNA replication. *Curr. Biol.* 16, 1571–1578. <https://doi.org/10.1016/j.cub.2006.06.068>.
244. Oomen, M.E., Fox, A.N., Gonzalez, I., Mollie, A., Papadopoulou, T., Pablo Navarro, P., and Dekker, J. (2023). Mitotic chromosomes harbor cell type and species-specific structural features within a universal looping

- architecture. Preprint at bioRxiv. <https://doi.org/10.1101/2023.12.08.570796>.
245. Das, M., Semple, J.I., Haemmerli, A., Volodkina, V., Scotton, J., Gitchev, T., Annan, A., Campos, J., Statzer, C., Dakhovnik, A., et al. (2024). Condensin I folds the *Caenorhabditis elegans* genome. *Nat. Genet.* 56, 1737–1749. <https://doi.org/10.1038/s41588-024-01832-5>.
 246. Fischle, W., Tseng, B.S., Dormann, H.L., Ueberheide, B.M., Garcia, B.A., Shabanowitz, J., Hunt, D.F., Funabiki, H., and Allis, C.D. (2005). Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature* 438, 1116–1122. <https://doi.org/10.1038/nature04219>.
 247. Banigan, E.J., and Mirny, L.A. (2019). Limits of Chromosome Compaction by Loop-Extruding Motors. *Phys. Rev. X* 9, 031007. <https://doi.org/10.1103/PhysRevX.9.031007>.
 248. Zhou, C.Y., Dekker, B., Liu, Z., Cabrera, H., Ryan, J., Dekker, J., and Heald, R. (2023). Mitotic chromosomes scale to nuclear-cytoplasmic ratio and cell size in *Xenopus*. *eLife* 12. <https://doi.org/10.7554/eLife.84360>.
 249. Choppakatla, P., Dekker, B., Cutts, E.E., Vannini, A., Dekker, J., and Funabiki, H. (2021). Linker histone H1.8 inhibits chromatin binding of condensins and DNA topoisomerase II to tune chromosome length and individualization. *eLife* 10, e68918. <https://doi.org/10.7554/eLife.68918>.
 250. Alavattam, K.G., Maezawa, S., Sakashita, A., Khouri, H., Barski, A., Kaplan, N., and Namekawa, S.H. (2019). Attenuated chromatin compartmentalization in meiosis and its maturation in sperm development. *Nat. Struct. Mol. Biol.* 26, 175–184. <https://doi.org/10.1038/s41594-019-0189-y>.
 251. Patel, L., Kang, R., Rosenberg, S.C., Qiu, Y., Raviram, R., Chee, S., Hu, R., Ren, B., Cole, F., and Corbett, K.D. (2019). Dynamic reorganization of the genome shapes the recombination landscape in meiotic prophase. *Nat. Struct. Mol. Biol.* 26, 164–174. <https://doi.org/10.1038/s41594-019-0187-0>.
 252. Rhodes, J.D.P., Haarhuis, J.H.I., Grimm, J.B., Rowland, B.D., Lavis, L.D., and Nasmyth, K.A. (2017). Cohesin Can Remain Associated with Chromosomes during DNA Replication. *Cell Rep.* 20, 2749–2755. <https://doi.org/10.1016/j.celrep.2017.08.092>.
 253. Zickler, D., and Kleckner, N. (1998). The leptotene-zygote transition of meiosis. *Annu. Rev. Genet.* 32, 619–697. <https://doi.org/10.1146/annurev.genet.32.1.619>.
 254. Cheng, G., Pratto, F., Brick, K., Li, X., Alleva, B., Huang, M., Lam, G., and Camerini-Otero, R.D. (2024). High resolution maps of chromatin reorganization through mouse meiosis reveal novel features of the 3D meiotic structure. Preprint at bioRxiv. <https://doi.org/10.1101/2024.03.25.586627>.
 255. Liang, Z., Zickler, D., Prentiss, M., Chang, F.S., Witz, G., Maeshima, K., and Kleckner, N. (2015). Chromosomes Progress to Metaphase in Multiple Discrete Steps via Global Compaction/Expansion Cycles. *Cell* 161, 1124–1137. <https://doi.org/10.1016/j.cell.2015.04.030>.
 256. Hirano, T. (2016). Condensin-Based Chromosome Organization from Bacteria to Vertebrates. *Cell* 164, 847–857. <https://doi.org/10.1016/j.cell.2016.01.033>.
 257. Yáñez-Cuna, F.O., and Koszul, R. (2023). Insights in bacterial genome folding. *Curr. Opin. Struct. Biol.* 82, 102679. <https://doi.org/10.1016/j.sbi.2023.102679>.
 258. Lioy, V.S., Courzac, A., Marbouty, M., Duigou, S., Mozziconacci, J., Es-péli, O., Boccard, F., and Koszul, R. (2018). Multiscale Structuring of the *E. coli* Chromosome by Nucleoid-Associated and Condensin Proteins. *Cell* 172, 771–783.e18. <https://doi.org/10.1016/j.cell.2017.12.027>.
 259. Pilatowski-Herzing, E., Samson, R.Y., Takemata, N., Badel, C., Bohall, P.B., and Bell, S.D. (2024). Capturing chromosome conformation in Crenarchaea. *Mol. Microbiol.* <https://doi.org/10.1111/mmi.15245>.
 260. Takemata, N., Samson, R.Y., and Bell, S.D. (2019). Physical and Functional Compartmentalization of Archaeal Chromosomes. *Cell* 179, 165–179.e18. <https://doi.org/10.1016/j.cell.2019.08.036>.
 261. Gornik, S.G., Hu, I., Lassadi, I., and Waller, R.F. (2019). The Biochemistry and Evolution of the Dinoflagellate Nucleus. *Microorganisms* 7, 245. <https://doi.org/10.3390/microorganisms7080245>.
 262. Rizzo, P.J. (2003). Those amazing dinoflagellate chromosomes. *Cell Res.* 13, 215–217. <https://doi.org/10.1038/sj.cr.7290166>.
 263. Ryba, T., Hiratani, I., Lu, J., Itoh, M., Kulik, M., Zhang, J., Schulz, T.C., Robins, A.J., Dalton, S., and Gilbert, D.M. (2010). Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. *Genome Res.* 20, 761–770. <https://doi.org/10.1101/gr.099655.109>.
 264. Klein, K.N., Zhao, P.A., Lyu, X., Sasaki, T., Bartlett, D.A., Singh, A.M., Tasan, I., Zhang, M., Watts, L.P., Hiraga, S.I., et al. (2021). Replication timing maintains the global epigenetic state in human cells. *Science* 372, 371–378. <https://doi.org/10.1126/science.aba5545>.
 265. Bulger, M., and Groudine, M. (1999). Looping versus linking: toward a model for long-distance gene activation. *Genes Dev.* 13, 2465–2477. <https://doi.org/10.1101/gad.13.19.2465>.
 266. Grosveld, F., van Staalduinen, J., and Stadhouders, R. (2021). Transcriptional Regulation by (Super)Enhancers: From Discovery to Mechanisms. *Annu. Rev. Genomics Hum. Genet.* 22, 127–146. <https://doi.org/10.1146/annurev-genom-122220-093818>.
 267. Tolhuis, B., Palstra, R.J., Splinter, E., Grosveld, F., and de Laat, W. (2002). Looping and Interaction between Hypersensitive Sites in the Active beta-globin Locus. *Mol. Cell* 10, 1453–1465. [https://doi.org/10.1016/s1097-2765\(02\)00781-5](https://doi.org/10.1016/s1097-2765(02)00781-5).
 268. Palstra, R.J., Tolhuis, B., Splinter, E., Nijmeijer, R., Grosveld, F., and de Laat, W. (2003). The beta-globin nuclear compartment in development and erythroid differentiation. *Nat. Genet.* 35, 190–194. <https://doi.org/10.1038/ng1244>.
 269. Deng, W., Lee, J., Wang, H., Miller, J., Reik, A., Gregory, P.D., Dean, A., and Blobel, G.A. (2012). Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. *Cell* 149, 1233–1244. <https://doi.org/10.1016/j.cell.2012.03.051>.
 270. Sanyal, A., Lajoie, B.R., Jain, G., and Dekker, J. (2012). The long-range interaction landscape of gene promoters. *Nature* 489, 109–113. <https://doi.org/10.1038/nature11279>.
 271. Hughes, J.R., Roberts, N., McGowan, S., Hay, D., Giannoulatou, E., Lynch, M., De Gobbi, M., Taylor, S., Gibbons, R., and Higgs, D.R. (2014). Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. *Nat. Genet.* 46, 205–212. <https://doi.org/10.1038/ng.2871>.
 272. Chen, L.F., Lee, J., and Boettiger, A. (2023). Recent progress and challenges in single-cell imaging of enhancer-promoter interaction. *Curr. Opin. Genet. Dev.* 79, 102023. <https://doi.org/10.1016/j.gde.2023.102023>.
 273. Du, M., Stitzinger, S.H., Spille, J.H., Cho, W.K., Lee, C., Hijaz, M., Quintana, A., and Cissé, I.I. (2024). Direct observation of a condensate effect on super-enhancer controlled gene bursting. *Cell* 187, 331–344.e17. <https://doi.org/10.1016/j.cell.2023.12.005>.
 274. Zuin, J., Dixon, J.R., van der Reijden, M.I.J.A., Ye, Z., Kolovos, P., Brouwer, R.W.W., van de Corput, M.P.C., van de Werken, H.J.G., van Knoch, T.A., van IJcken, W.F.J., et al. (2014). Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. *Proc. Natl. Acad. Sci. USA* 111, 996–1001. <https://doi.org/10.1073/pnas.1317788111>.
 275. Xiao, J.Y., Hafner, A., and Boettiger, A.N. (2021). How subtle changes in 3D structure can create large changes in transcription. *eLife* 10, e64320. <https://doi.org/10.7554/eLife.64320>.
 276. Calderon, L., Weiss, F.D., Beagan, J.A., Oliveira, M.S., Georgieva, R., Wang, Y.F., Carroll, T.S., Dharmalingam, G., Gong, W., Tossell, K., et al. (2022). Cohesin-dependence of neuronal gene expression relates to chromatin loop length. *eLife* 11, e76539. <https://doi.org/10.7554/eLife.76539>.

277. Adams, N.M., Galitsyna, A., Tiniakou, I., Esteva, E., Lau, C.M., Reyes, J., Abdennur, N., Shkolikov, A., Yap, G.S., Khodadadi-Jamayran, A., et al. (2024). Cohesin-mediated chromatin remodeling controls the differentiation and function of conventional dendritic cells. Preprint at bioRxiv. <https://doi.org/10.1101/2024.09.18.613709>.
278. Kane, L., Williamson, I., Flyamer, I.M., Kumar, Y., Hill, R.E., Lettice, L.A., and Bickmore, W.A. (2022). Cohesin is required for long-range enhancer action at the Shh locus. *Nat. Struct. Mol. Biol.* 29, 891–897. <https://doi.org/10.1038/s41594-022-00821-8>.
279. Guckelberger, P., Doughty, B.R., Munson, G., Rao, S.S.P., Tan, Y., Cai, X.S., Fulco, C.P., Nasser, J., Mualim, K.S., Bergman, D.T., et al. (2024). Cohesin-mediated 3D contacts tune enhancer-promoter regulation. Preprint at bioRxiv. <https://doi.org/10.1101/2024.07.12.603288>.
280. Chen, H., Levo, M., Barinov, L., Fujioka, M., Jaynes, J.B., and Gregor, T. (2018). Dynamic interplay between enhancer-promoter topology and gene activity. *Nat. Genet.* 50, 1296–1303. <https://doi.org/10.1038/s41588-018-0175-z>.
281. Hnisz, D., Weintraub, A.S., Day, D.S., Valton, A.L., Bak, R.O., Li, C.H., Goldmann, J., Lajoie, B.R., Fan, Z.P., Sigova, A.A., et al. (2016). Activation of proto-oncogenes by disruption of chromosome neighborhoods. *Science* 351, 1454–1458. <https://doi.org/10.1126/science.aad9024>.
282. Rekaik, H., and Duboule, D. (2024). A CTCF-dependent mechanism underlies the Hox timer: relation to a segmented body plan. *Curr. Opin. Genet. Dev.* 85, 102160. <https://doi.org/10.1016/j.gde.2024.102160>.
283. Kiefer, L., Gaudin, S., Rajkumar, S.M., Servito, G.I.F., Langen, J., Mui, M.H., Nawsheen, S., and Canzio, D. (2024). Tuning cohesin trajectories enables differential readout of the *Pcdh α* cluster across neurons. *Science* 385, eadm9802. <https://doi.org/10.1126/science.adm9802>.
284. Canzio, D., and Maniatis, T. (2019). The generation of a protocadherin cell-surface recognition code for neural circuit assembly. *Curr. Opin. Neurobiol.* 59, 213–220. <https://doi.org/10.1016/j.conb.2019.10.001>.
285. Flavahan, W.A., Drier, Y., Liau, B.B., Gillespie, S.M., Venteicher, A.S., Stemmer-Rachamimov, A.O., Suvà, M.L., and Bernstein, B.E. (2016). Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature* 529, 110–114. <https://doi.org/10.1038/nature16490>.
286. Kim, K.L., Rahme, G.J., Goel, V.Y., El Farran, C.A., Hansen, A.S., and Bernstein, B.E. (2024). Dissection of a CTCF topological boundary uncovers principles of enhancer-oncogene regulation. *Mol. Cell* 84, 1365–1376.e7. <https://doi.org/10.1016/j.molcel.2024.02.007>.
287. Peters, J.M. (2021). How DNA loop extrusion mediated by cohesin enables V(D)J recombination. *Curr. Opin. Cell Biol.* 70, 75–83. <https://doi.org/10.1016/j.ceb.2020.11.007>.
288. Zhang, Y., Zhang, X., Dai, H.Q., Hu, H., and Alt, F.W. (2022). The role of chromatin loop extrusion in antibody diversification. *Nat. Rev. Immunol.* 22, 550–566. <https://doi.org/10.1038/s41577-022-00679-3>.
289. Ba, Z., Lou, J., Ye, A.Y., Dai, H.Q., Dring, E.W., Lin, S.G., Jain, S., Kyritsis, N., Kieffer-Kwon, K.R., Casellas, R., and Alt, F.W. (2020). CTCF orchestrates long-range cohesin-driven V(D)J recombinational scanning. *Nature* 586, 305–310. <https://doi.org/10.1038/s41586-020-2578-0>.
290. Dai, H.Q., Hu, H., Lou, J., Ye, A.Y., Ba, Z., Zhang, X., Zhang, Y., Zhao, L., Yoon, H.S., Chapdelaine-Williams, A.M., et al. (2021). Loop extrusion mediates physiological IgH locus contraction for RAG scanning. *Nature* 590, 338–343. <https://doi.org/10.1038/s41586-020-03121-7>.
291. Zhang, Y., Zhang, X., Ba, Z., Liang, Z., Dring, E.W., Hu, H., Lou, J., Kyritsis, N., Zurita, J., Shamim, M.S., et al. (2019). The fundamental role of chromatin loop extrusion in physiological V(D)J recombination. *Nature* 573, 600–604. <https://doi.org/10.1038/s41586-019-1547-y>.
292. Hill, L., Ebert, A., Jaritz, M., Wutz, G., Nagasaka, K., Tagoh, H., Kostanova-Poliakova, D., Schindler, K., Sun, Q., Bönel, P., et al. (2020). Wapl repression by Pax5 promotes V gene recombination by IgH loop extrusion. *Nature* 584, 142–147. <https://doi.org/10.1038/s41586-020-2454-y>.
293. Owen, J.A., Osmanović, D., and Mirny, L. (2023). Design principles of 3D epigenetic memory systems. *Science* 382, eadg3053. <https://doi.org/10.1126/science.adg3053>.