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REVIEW ARTICLE

Physical mechanisms of chromatin spatial organization

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In higher eukaryotes, chromosomes have a complex three-dimensional (3D) conformation in the cell nucleus serving vital functional purposes, yet their folding principles remain poorly understood at the single-molecule level. Here, we summarize recent approaches from polymer physics to comprehend the physical mechanisms underlying chromatin architecture. In particular, we focus on two models that have been supported by recent, growing experimental evidence, the Loop Extrusion model and the Strings&Binders phase separation model. We discuss their key ingredients, how they compare to experimental data and some insight they provide on chromatin architecture and gene regulation. Progresses in that research field are opening the possibility to predict how genomic mutations alter the network of contacts between genes and their regulators and how that is linked to genetic diseases, such as congenital disorders and cancer.

Introduction

New high-throughput technologies based on sequencing, such as Hi-C, and super-resolution microscopy [1,2] are providing detailed, quantitative information about the architecture of the genome and, in particular, on the network of interactions formed by regulatory regions and their target genes [3–5]. Strong contact loops are found genome-wide between single pairs of distal DNA sites such as genes and enhancers [6]. Chromatin is also structured into a sequence of megabase sized regions, named Topological Associating Domains (TADs) [7,8], marked by strong self-interactions, which are thought, e.g., to confine the activity of enhancers to their proper targets while TAD boundaries act as spatially insulating structures. A/B compartments have also been discovered, i.e., domains of active and repressed chromatin having a size in the range of tens of Mbs [9]. Additionally, complex architectural patterns exist both at the sub-TAD level [10] and at larger scales, as TADs form higher-order structures (meta-TADs) [11] arranged in a hierarchy of domains-within-domains across genomic scales up to encompassing A/B compartments and entire chromosomes. The organization of the genome inside the nucleus typically involves multiple contacts, e.g., triplets, between distal regions such

Abbreviations
CTCF, CCCTC-binding factor; LE, Loop Extrusion model; MLL3/4, mixed-lineage leukemia protein ¾; Pol-II, RNA polymerase II; Pol-II-S2p, RNA polymerase II phosphorylated at Ser2; PRC2, Polycomb repressive complex 2; SBS, Strings&Binders model; TAD, topological associating domains.

as super-enhancers [12], hubs of interchromosomal interactions as those formed around the nucleolus or nuclear speckles [13], and interactions with the nuclear lamina where hundreds of large, gene repressive domains (named LADs) are formed [14]. Importantly, it has been discovered that large genomic mutations, in particular in noncoding regions, can interfere with the correct folding of DNA and, hence, alter the physical contacts between genes and their regulatory elements, thus resulting in severe human diseases, such as congenital disorders [15] and cancers [16,17]. Yet, the physical and molecular mechanisms shaping those contacts and controlling the functioning of the genome remain largely mysterious.

Here, we review the basic aspects of some of the quantitative models introduced from polymer physics to comprehend the physical mechanisms determining chromatin folding. We focus in particular on the Loop Extrusion model and the Strings&Binders phase separation model, and the scenario they depict of chromatin organization and gene regulation.

Chromatin organizing factors

Among the factors involved in the 3D organization of chromatin, CTCF binding sites and cohesin have been associated with the formation of loops and TADs [6] and linked to loop extrusion mechanisms [18–20]. Depletion of CTCF or cohesin leads indeed to loop release in bulk Hi-C data, albeit interactions signals persist at the compartment level and within former loops or TADs [21–23]. As mentioned, compartments A and B correlate to different transcriptional states [9], and homotypic interactions between active and poised gene promoters, associated, respectively, with Pol-II-S2p and PRC2, have been observed at the Mb scale and linked to phase separation mechanisms [24,25]. Indeed, physical mechanisms of phase separation are becoming a paradigm of cell organization [26,27] and of transcriptional control [28], as Pol-II, transcription factors, and coactivators, such as Mediator, have been shown to form condensates [29–32] involved in gene regulation [28,33–35].

Chromosomal contacts and TADs have a strong variability from cell to cell, as revealed by single-cell Hi-C experiments [36–39]. Additionally, multiplexed FISH microscopy approaches have shown that, while TAD-like globular 3D chromatin structures are present at the single-molecule level in single-cells, they are broadly varying from cell to cell [40–43]: for example, TAD boundaries can occur with nonzero probability at any genomic location and are enriched only at a subset of CTCF sites in the considered regions [42], hinting that chromatin contacts could arise from mechanisms different from the loop extrusion.

Models of chromatin architecture from polymer physics

To make sense of the complexity of chromatin interaction data and explain the mode of action of their underlying molecular factors, two main models from polymer physics have been introduced to-date that are supported by growing experimental evidence. Here, we briefly review the key ingredients of those models, how they compare to experimental data and the emerging picture of the physical mechanisms underlying chromatin spatial organization.

The Loop Extrusion model (Fig. 1A) envisages that a molecular complex acts as an active motor extruding DNA loops between cognate anchor points, in a nonequilibrium process requiring energy influx by, e.g., ATP molecule consumption [18,19,44]. A different scenario, recapitulated by the Strings&Binders model (Fig. 1B), posits that chromatin interactions are mediated by diffusing cognate binding molecules, such as transcription factors (TFs), that can bridge pairs (or multiplets) of DNA sites via mechanisms of equilibrium polymer thermodynamics [24,26,45–60]. In such a scenario, DNA-molecule interactions induce chromatin structural changes via thermodynamics phase transitions, such as coil-to-globule or phase separation transitions, which spontaneously establish contacts or segregate specific, distal DNA sites, such as genes and enhancers.

It is also worth mentioning important computational approaches to reconstruct chromosome 3D conformations independent of the underlying physical processes, based on the optimization of scoring functions that compare contact data and inferred model 3D structures, albeit, for brevity, they are not discussed below [61–74].

The Loop Extrusion model

The core idea behind the *Loop Extrusion* (LE) Model [18,19,44] is that a loop-extruding factor, assumed to be cohesin, binds on DNA and actively extrudes a chromatin loop up to reach its extrusion blocking sites envisaged to be CTCF binding sites of opposite orientation (Fig. 1A). Eventually, the extrusion complex can dissociate from DNA and release the loop. The model details can be found in recent reviews [75]. From a physics point of view, the LE model describes an off-equilibrium process where an active motor (cohesin) burns energy, such as ATP, to extrude

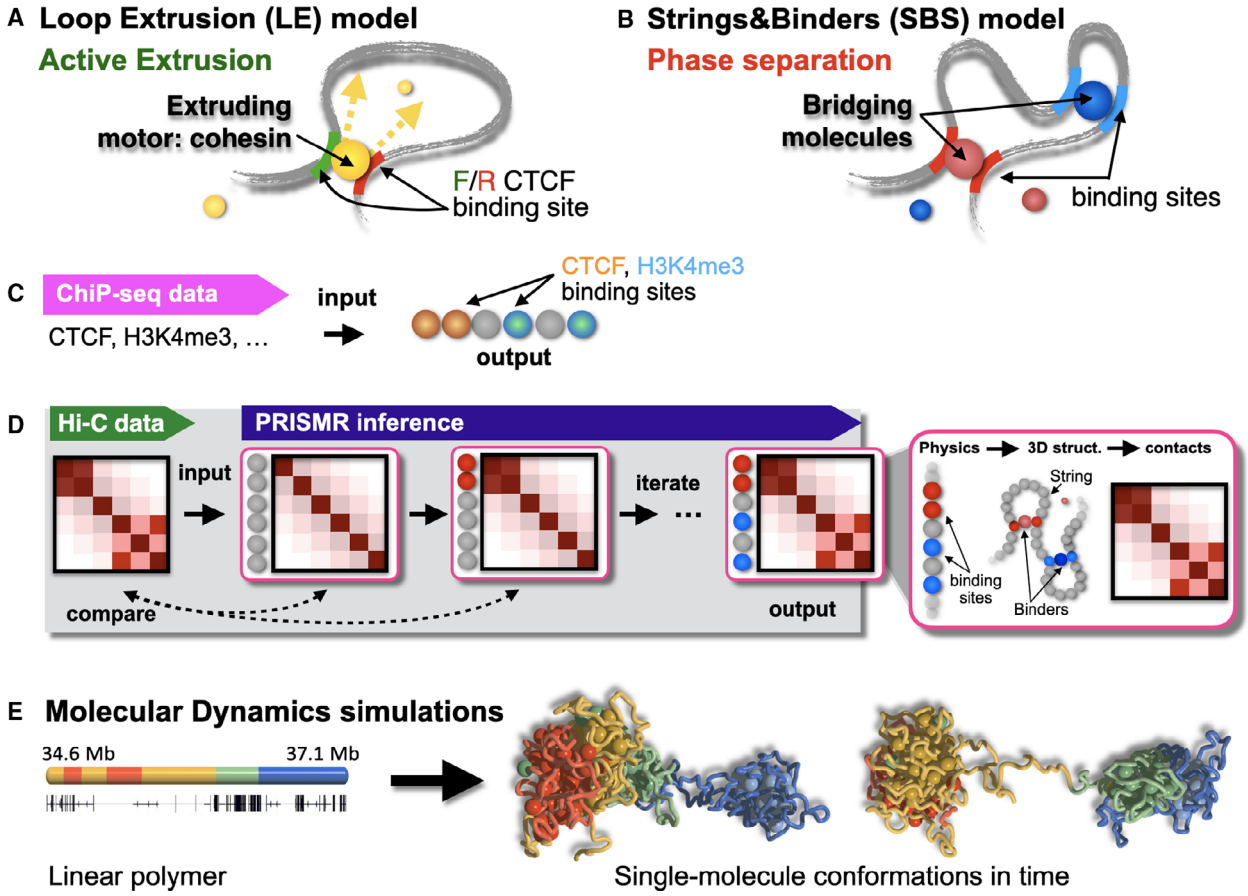

Fig. 1. Polymer physics models of chromatin. (A) The Loop Extrusion (LE) model poses that a cohesin complex acts as an active motor extruding chromatin loops, whose anchor points along DNA are pairs of CTCF binding sites of opposite orientation [18,19,44]. A variant of the LE, the Slip-Link model [20], posits that cohesin becomes loaded at adjacent pairs of sites on the DNA chain and each of those sites can randomly slide, hence growing a loop, up to the anchor sites, with no energy inputs. The LE model is supported by a variety of important observations, and many experimental results can be successfully interpreted. (B) The Strings&Binders (SBS) model of chromatin considers the scenario where contacts between distal DNA regions are established by diffusing cognate binding factors [24,26]. A chromatin filament is modeled as a polymer chain and along the chain are located binding sites for different diffusing molecular binders. In the SBS model, chromatin contact patterns are established by a thermodynamics mechanism of globule phase separation. It has been shown to explain Hi-C, GAM, and FISH data across chromosomal scales and cell types [24,25,49,57] and has been validated by predicting the impact of disease-linked mutations on the 3D structure of DNA [46,93,97]. (C) The genomic location of the binding sites of the model of a locus of interest can be derived by epigenetic and TF Chip-seq data. (D) Alternatively, they can be inferred by a Machine Learning procedure (PRISMR, [46]) that searches the minimal set of binding sites that, based only on physics, folds the polymer to best match input bulk Hi-C data. (E) An ensemble of single-molecule 3D structures of chromatin can be derived by Molecular Dynamics computer simulations from the inferred linear polymer model of the investigated loci [88]. Computer simulations also permit to access the real time dynamics of chromatin, e.g., how contact patterns are established and change in time or under different conditions.

chromatin loops. The *Slip-Link* model is a variant of the LE where extrusion occurs driven by thermal diffusion, without requiring an energy burning motor [20]. Various choices of the model different parameters can reproduce patterns of contact data beyond point-wise interactions between the loop anchor points, such as TADs and lines of enriched contacts between a CTCF site and a flanking region, features typically visible in Hi-C data.

The loop extrusion model has been invoked to explain experiments, for instance, on mitotic chromosome compaction and segregation [76], meiotic chromosome organization in *S. cerevisiae* [77] and V(D)J recombination [78,79].

Importantly, a direct experimental observation of the motor activity of factors such as condensin and cohesin in extruding DNA loops has been recently provided by *in vitro* single-molecule experiments, albeit in simplified conditions, giving evidence in favor of the loop extrusion mechanism [80–83]. In addition to giving a natural interpretation to the CTCF convergence bias in loops visible in Hi-C data, indirect support of the LE model is also found in important experiments where perturbation of CTCF or cohesin affect chromatin organization. For example, disruption of specific CTCF binding sites produces architectural rearrangements in agreement with LE [18,84,85]. Also, genome-wide cohesin or CTCF degron leads to massive disappearance of TADs and loops [21–23,86].

However, bulk Hi-C data produced in CTCF or cohesin depletion experiments reveal also that interactions persist at the A/B compartment level and within former loops or TADs [21–23]. Additionally, multiplexed FISH microscopy has shown that in single-cells TAD-like domains are broadly varying [40–43] and TAD boundaries can occur with nonzero probability at any genomic location, not just at a subset of CTCF sites [42]. Those important experiments provide evidence that chromatin 3D architecture is only partially dependent on CTCF/cohesin and arises also from mechanisms different from the loop extrusion.

The Strings&Binders model

Another class of polymer models of chromatin architecture has explored the picture where specific interactions exist between different types of distal DNA binding sites, either arising by direct contact or established by diffusing molecules, such as transcription factors (TFs), that bridge those sites, hence producing DNA loops [24,26,45–60]. Those models investigate the emergent structural properties of the system, derived by polymer thermodynamics, and form a broad class of universality, as dictated by Statistical Mechanics. Here we focus on a well-known example within this class, the *Strings&Binders* model (SBS) [26] (Fig. 1B), which has been broadly applied to investigate chromatin structure at the single-molecule level in wild-type genomes and to understand the impact of disease-associated mutations. In the SBS model, a chromatin filament is represented as a self-avoiding-walk polymer having specific as well as unspecific binding sites for cognate, diffusing molecular binders [24,26]. Driven by thermodynamics, above a threshold concentration the binders stably bridge their cognate sites, thus forming loops and defining the system architecture.

The core idea of the SBS model is that, as dictated by polymer physics [87], the system equilibrium 3D conformations fall in just a few folding classes corresponding to its thermodynamics phases, which can be predicted by physics. For example, as the number of binders (or affinity strength) grows above a threshold point, the system typically undergoes a phase transition from a coil, randomly folded state to a globular, more compact state. Hence, by determining the system thermodynamics phases one can derive the full ensemble of 3D conformations where it spontaneously folds into. Note that in a given thermodynamic state, i.e., for a given binder concentration, the system can fold in a variety of 3D conformations, not just in a unique, naive structure, so resulting in a broad variability of single-molecule architectures. The model details can be found in recent reviews [88,89].

To derive the specific architecture of a genomic region of interest, it is necessary to identify the specific genomic location and the types of the binding sites of the polymer model of such a region (Fig. 1C, D). A typical approach exploits prior knowledge of epigenetic or TFs Chip-seq signals (Fig. 1C). Indeed, a number of molecular factors has been discovered to have a role in chromosome architecture, encompassing a variety of TFs and epigenetic tracks, such as CTCF/Cohesin [18], MLL3/4 [90], polycomb repressive complex 1 [91], active and poised Pol-II [25]. The model is informed with such known binding sites and, next, its 3D conformations are derived by Molecular Dynamics simulations (Fig. 1E) [45,47–49,51,56–58,60]. The advantage of such an approach is that different scenarios can be tested to understand the nature of the key factors shaping the architecture, yet a limitation is that only known factors can be considered. A different approach (Fig. 1D) exploits a machine learning procedure whereby from only contact data, say Hi-C data, the minimal set of putative model binding sites is inferred which best explains, out of only physics, the input data with no additional prior knowledge [46,50,59]. Next, to learn the molecular nature of the inferred model binding sites, their genomic position is correlated with available information on chromatin organizing factors in the same cell type. Note that usually a single binding site type must not be identified with a single molecular factor; conversely, it has been shown that different binders typically correlate with distinct combinations of factors [45]. Such a method is advantageous to avoid biases toward a subset of TFs in explaining contact data and to discover novel combinations of molecular elements or new putative factors that control folding.

The SBS model has been used to understand the 3D conformations of a number of loci, such as the HoxB

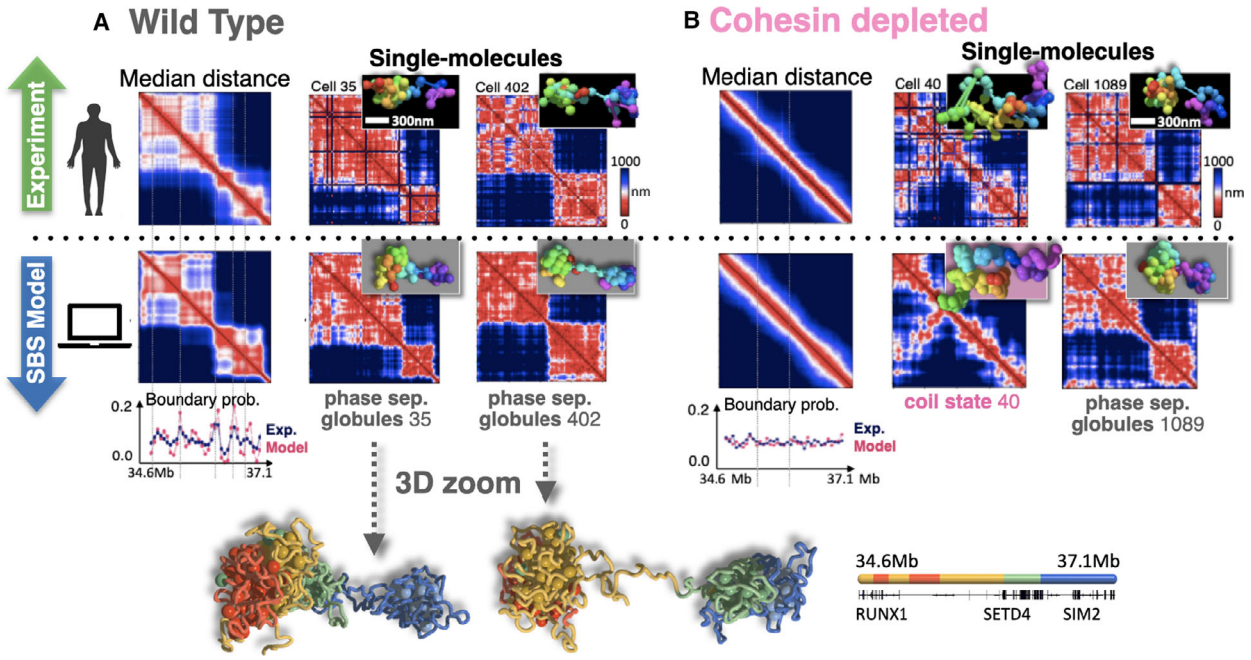

Fig. 2. Single-molecule 3D structures derived by globule phase separation within the SBS model can explain single-cell multiplexed FISH microscopy data. (A) The single-molecule conformations derived by SBS model of a human locus in HCT116 cells [50] match very well single-cell microscopy data from multiplexed FISH experiments [42]. The mechanism underlying globule formation was traced back to polymer phase separation, whereas the variability of single-molecule conformations results from the intrinsic degeneracy of the system thermodynamic microstates. (B) The model comparison with experimental data of the same locus in cohesin depleted cells shows that phase separation is reversed back into random coil conformations, erasing average patterns [50]. Interestingly, recent experiments have shown that cohesin does phase separates into aggregates with DNA in an ATP-independent manner [52].

[25], HoxD [92], Shh [93], or alpha/beta-globin locus [94]. It has also been employed to shed light on the architectural rearrangements upon differentiation of those loci, as cell type- and gene-specific multiway contacts are established with regulatory elements in connection to epigenetic and transcriptional changes [25,92,94,95]. Models of interacting polymers, in the same class of the SBS, have been successfully employed to explain TAD and contact pattern formation also at chromosomal scales [45,48,57,96], to explore structural heterogeneity at the single-molecule level [47,51], and to dissect Hi-C data in a variety of loci, chromosomes and organisms, such as yeast, Drosophila, murine, and human cells [40,56,58]. A limitation of this type of models is that they need additional ingredients to explain the CTCF convergence bias of loops, which is instead naturally included within the LE framework. In this direction, models combining both LE and affinity-based (e.g. SBS) mechanisms have been shown to describe well chromatin folding data [21,47,49].

The SBS model has been also important to investigate the mechanisms underlying the formation of TADs at the single-molecule level (Fig. 2) in a variety of specific loci [25,59], and its predictions have been validated against single-cell imaging data [50]. Those studies have provided evidence that chromatin TAD-like globules, revealed by microscopy experiments (see, e.g., [42]), are established by a thermodynamics mechanism of polymer phase separation [50]. The distinct globules self-assemble by the combinatorial action of different chromatin organizing factors, including, but not limited to CTCF and cohesin. Those globules define stable environments where specific contacts between cognate regions (e.g., gene-enhancers) are favored over stochastic encounters. That is a robust, reversible mechanism of spatial organization, where stochasticity and specificity co-exist. In particular, the broad cell-to-cell variability of 3D structures naturally emerges from the thermodynamic degeneracy of conformations predicted by the theory. Applications to cohesin depleted cells have shown that cohesin depletion reverses phase separation into randomly folded states, hence erasing average interaction patterns [50], in agreement with recent experiments that have confirmed that cohesin shows pronounced clustering on

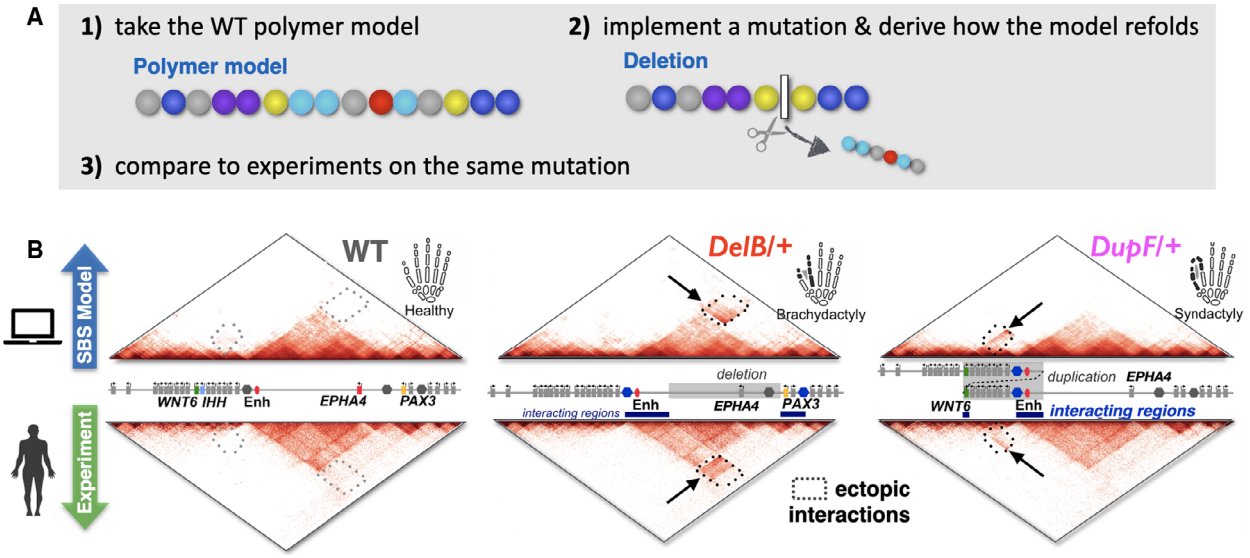

Fig. 3. The SBS model predicts how structural variants rewire gene-enhancers contacts. (A) The SBS model of a genomic region can be used to make predictions on the impact of large mutations, such as structural variants, on the locus architecture and, in particular, on the rewiring of regulatory contacts between genes and enhancers (enhancer-hijacking). (B) The shown examples concern the human EPHA4 locus, where different mutations are associated with different limb malformations. The SBS model predictions are all confirmed by independent cHi-C experiments, providing insights on the mechanisms whereby ectopic gene activation is induced and the phenotype developed [46].

DNA, in an ATP-independent manner, typical of phase separation [52].

The predictive power of the SBS model to reconstruct the impact on the 3D architecture of large mutations (Structural variants) linked to human diseases has been successfully tested against experiments in different cell types and loci, such as the human EPHA4 [46], Pitx1 [97], and Shh [93] loci. In particular, the model was used to predict how those mutations rewire the contacts between genes and their regulators (*enhancer-hijacking*) hence activating ectopic transcription that leads to disease, and how different mutations induce distinct enhancer-hijackings and phenotypes (Fig. 3). It was also used to show how 3D conformation determines enhancer tissue specificity and morphogenic identity [97].

Concluding remarks and perspectives

As the wealth and complexity of experimental data are growing, models from polymer physics are becoming essential to dissect the mechanisms underlying chromosome spatial organization and its functional implications. We focused, in particular, on the scenarios depicted by the Loop Extrusion and Strings&Binders polymer models, which are supported by a number of recent experiments.

DNA loop extrusion has emerged as an important mechanism of chromatin organization [75], posing that a cohesin linked active motor extrudes loops between CTCF anchor points, in a nonequilibrium, active process. The extruding motor activity of cohesin has been recently also experimentally confirmed *in vitro* [81,82] and its role in chromatin architecture supported by bulk Hi-C data in systems depleted for CTCF or cohesin [21–23]. However, super-resolution single-cell imaging experiments in human loci have shown that DNA interactions can arise from other important molecular process [42], consistent with a folding mechanism based on polymer phase separation as depicted by the Strings&Binders model [24,50]. Intriguingly, novel experiments in yeast have shown that cohesin also phase separates into aggregates with DNA in an ATP-independent manner [52].

The models we discussed appear to return a simplified description of the molecular complexity of real chromatin, yet they may capture real features of chromosome folding because of the Statistical Mechanics concept of universality in phase transitions [87], whereby stylized models can exhibit the same emergent features of their more detailed and refined counterparts. Yet, more faithful molecular representations of chromatin can reveal a variety of additional specific properties, which could be relevant

to different biological situations. Additionally, it remains to be clarified under which circumstances loop extrusion can proceed on real chromatin *in vivo* in the complex environment of the nucleoplasm or, within the SBS model scenario, how near equilibrium can be reached.

Nevertheless, the Loop Extrusion model appears to be a basic chromatin organizational mechanism, which can be implemented by active motors as well as by diffusion, as in its Slip-Link variant. And thermodynamics phase transitions and self-assembling, as those described by the Strings&Binders model, are reliable and reversible mechanisms to control conformations, requiring no energy input beyond the thermal bath. Importantly, phase transition mechanisms require no molecular fine-tuning as the system can be transited in a different structural phase by basic cell processes, such as up-regulation of TFs or epigenetic factors [26]. However, other folding mechanisms, yet to be discovered, are likely to play a role in establishing chromatin architecture, and in different chromosomal regions, different physical processes could contribute or co-exist.

Importantly, models from polymer physics are providing a deeper understanding of the 3D organization of the genome and how it is altered by mutations linked to phenotypes, relevant to congenital disorders [15] or cancer [16,17,98]. In this regard, recent analysis on thousands of cancer genomes [99] involving several tumor types, identified structural variation as one of the key mutational processes in cancer [100] highlighting even more the deep connection between chromatin 3D architecture and disease. Hence, the strategic combination of quantitative models and advanced experimental technologies can help opening new routes to design strategies to attack diseases linked to genomic architectural modifications.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

AMC, SB, and MN wrote the manuscript with inputs from the other authors.

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