

REVIEW ARTICLE

Pitfalls and missing links in current understanding of 4D genomes

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Abstract

The spatial and temporal organization of the genome—collectively termed the 4D genome—is pivotal for regulating gene expression, maintaining genome stability, and guiding development. The convergence of chromosome conformation capture technologies, super-resolution microscopy, and single-cell epigenomics has transformed our understanding of chromatin structure and function. Despite these advances, critical gaps remain, particularly, in integrating multi-scale data, interpreting dynamic cellular states, and bridging evolutionary and developmental processes. This critical review synthesizes recent findings and identifies three key conceptual pitfalls: scale transformation, temporal causality, and plasticity in evolutionary-developmental dynamics. I also outline emerging methodologies and theoretical frameworks aimed at overcoming these challenges and propose directions for future research to achieve a unified and predictive model of a functional genome.

KEYWORDS

4D genome biology, chromatin structure and dynamics, evo-devo, systems biology

1 | INTRODUCTION

Understanding the spatial and temporal organization of the genome—the so-called 4D genome (1D refers to primary sequence loci, 2D refers to locus-locus contact/interaction, 3D refers to spatial conformation)—is a central objective in modern genome biology [1]. Chromatin conformation not only reflects transcriptional programs but also regulates access to genomic information and ensures genome stability. Advances, such as ChIP-seq, ATAC-seq, Hi-C, ChIA-PET, and imaging modalities like nanoFISH and OligoSTORM, have facilitated the mapping of genome structure and interactions with increasing resolution. However, our current models often remain fragmented, with lack of integration between scales (from nucleosomes to nuclear territories), along

time points (e.g., during mitosis or stress or embryonic development), and across developmental or evolutionary contexts. Here, I identify three critical gaps—scale integration, dynamic regulation, and developmental/evolutionary plasticity—that hinder our ability to form a holistic view of the 4D genome.

2 | MULTI-SCALE CHROMATIN ARCHITECTURE: BRIDGING PHYSICAL AND FUNCTIONAL DOMAINS AND CONNECTING HIERARCHICAL LEVELS

At the smallest scale, DNA wraps around histone proteins to form nucleosomes [2] (Figure 1). These are arranged in higher-order structures; traditionally in vitro

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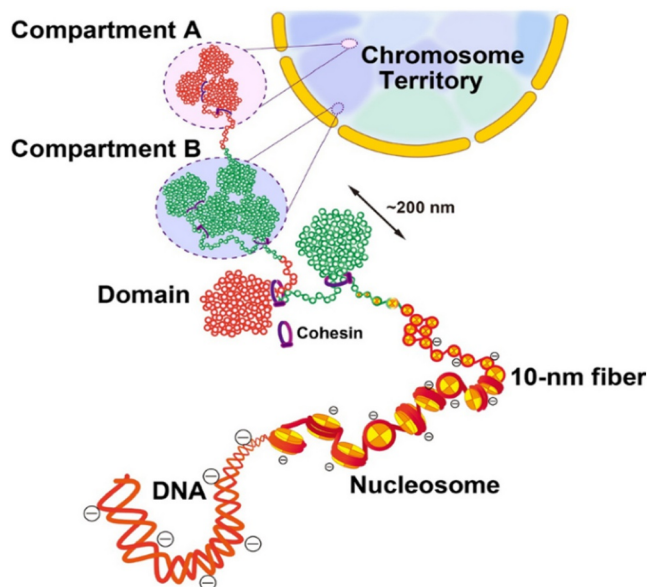


FIGURE 1 Hierarchical 3D genome organization [2].

studies showed that depending on the extent of cation-dependent DNA charge neutralization, nucleosome arrays can exist in an extended 10-nm conformation, a folded 30-nm fibers, or as phase-separated condensates. However, a recent study on the 3D architecture of native chromatin fibers in intact interphase human T-lymphoblasts using Cryo-FIB/Cryo-ET has challenged this model, suggesting that uniform 30-nm fibers do not exist *in vivo*; instead, a more disordered, flexible, and relaxed dynamic chromatin state exists [3]. Chromatin seems solid on the mesoscale and behaves like a liquid on the nanoscale. Can traditional statistical mechanical models, such as spin-glass model or polymer physics model, be really applied to biological living systems?

At the mesoscale, chromatin forms hierarchical domains such as loops, and topologically associating domains (TADs, around 1 Mb), reportedly governed by loop extrusion mechanisms mediated by cohesin and condensin complexes, and large A/B compartments, largely correlated to gene transcriptional activities. Although the development of high-throughput sequencing technologies (e.g., Hi-C, ChIP-PET) and imaging-based assays for 3D genome mapping (e.g., MERFISH) has substantially advanced our understanding of chromatin organization, comparison or reconciliation between sequencing and imaging results has proven extremely difficult for a variety of reasons, including different methodologies, different samples or conditions, and ensemble average versus single cell measurement. For example, regarding the interplay between chromatin structure and DNA replication, the conventional view is that early DNA replication predominantly occurs within active gene-rich A compartments, whereas late replication is associated with repressed, compact B compartments. Furthermore, early replication

events (i.e., active origin of replications) often occur at TAD boundary regions. However, EdU pulse-labeling and chase, together with 3D-SIM [4], showed that there was a relative under-representation of EdU-labeled DNA in low DNA density classes, representing the active nuclear compartment, and an over-representation in high density classes representing the inactive nuclear compartment. It is very likely that Hi-C and many related sequencing-based contact mapping methods are more biased toward the active euchromatin and low DNA density regions, whereas over 50% genome is made of repetitive DNA forming heterochromatin. A scEdU-seq study [5] reveals that DNA replication speed in H3K9me3-repressed chromatin domains is actually faster than regions with active histone marks, where transcription-inflicted DNA damage impedes replication in early S phase. These studies underscore that our understanding of heterochromatin replication in eukaryotes is currently incomplete, which can be attributed to the current sequencing information that is more skewed toward euchromatic regions containing more efficient origins, methods that require DNA fragmentation and PCR amplification for library preparation, or the inherent limitation of short-read mapping problems. Another limitation of many current loop models is based on homogeneous polymer biophysics models. By definition, these models lack sequence and cell-type specificity. Mapping only to the reference genome is also problematic practice, especially for unstable cells (e.g., cancer cells). Sequence variations (e.g., translocations, DNA circles, chromothripsis, etc.) and epigenomic conformation changes (e.g., unaccounted for by sequence-only based contact prediction models) are likely missed by such modeling and mapping strategies.

At the nuclear scale, entire interphase chromosomes occupy discrete territories (i.e., chromosomal territories CT, partitioned into compartments TADs and loops), organized with respect to nuclear bodies (NBs) and the lamina (e.g., LADs, around 1 Mb). The image of interphase NBs can be found on the Lawrence Lab's website at UMass Chan Medical School. NBs, such as the nucleolus, Cajal bodies (CajB), and nuclear speckles, are membrane-less organelles formed by phase separation. They play critical roles in organizing transcriptional and post-transcriptional machinery, anchoring regulatory regions, and shaping chromatin compartments. Often changes can be easily seen at nuclear imaging or cytogenetic levels, but are difficult to see at the TAD level, partly due to TAD's relatively stable and conserved character. Therefore, spatially localizing and integrating various 3D genome domains to different functional subnuclear structures is urgently needed.

For example, human nucleoli comprise the fibrillar center (FC)/granular component (GC)/nucleolar rim (NR) multilayered structures. The detailed explanatory image is shown in Ref. [6]. Active RNA polymerase

(RNAP) I transcription occurs in the FC core and silenced chromatin loci, including Xi, pericentromeric regions, and inactive ribosomal DNA copies, are sequestered at NR. Human rDNA tandem copies (clusters in acrocentric chromosomes 13, 14, 15, 21 and 22) comprise the transcribed regions and are separated by intergenic spacer (IGS) sequences. Their structure and function can be studied by modern nucleolar DNA spatial omics methods such as nHi-C (nucleolus-associated chromatin interactions) and Nucleolar DamID [6]. Such studies suggest that NBs not only provide spatial scaffolds but also act as assemblers that regulate chromatin accessibility and insulation.

Both principled and realistic mathematical models are ultimately required, as nature only speaks the language of mathematics. Constrained polymer physics, combined with numerical molecular dynamics simulation and machine learning-based inference, has been successful in modeling Hi-C/FISH patterns and local chromatin folding (e.g., loop-extrusion and polymer phase-separation) (e.g., Refs. [7, 8]). Hierarchical loops and TADs are topological structures. Decomposing 3D genome structures by topological invariance (e.g., cohomology theory, Hodge theory, etc.) and building “Periodic Table” to classify all loops by topological data analysis (TDA or topological persistent analysis), as initiated by single-molecule localization microscopy (SMLM, see Figure 2 and Ref. [9]), are certainly very appealing.

Despite these hierarchical models, the major challenge lies in integrating observations across these scales. Two key points must be emphasized: (1) Heterogeneity—sequence based or polymer models—must allow positional and epigenetic variation. Spatial single-cell omics may be required for demixing and rare structure detection. (2) Transformation and connection laws between adjacent layers need to be worked out; the best approach is top-down, not bottom-up (because biological system is “Goal” oriented). Driven by AI for science, we are expecting “Chromatin Alpha-fold” or “Alpha-3DGenome” to appear perhaps in the not-too-distant future.

3 | CHROMATIN DYNAMICS: HOW 3D GENOME STRUCTURE ARE REFORMED OR TRANSFORMED

All biological structures are dynamic, and stability of a structure and transition between structures depends on the timescales. For example, NB structures serve as dynamic hubs that interact with specific genomic loci in a context-dependent manner. It is well-known that active transcription factories are often in proximity to nuclear speckles enriched in RNA polymerase II and splicing factors. However, our knowledge on how 3D genome and their associated NBs are assembled or

disassembled is very limited, even during the most fundamental cellular dynamics: (mitotic or meiotic) cell cycle and cellular stress response.

Although mitotic chromatin Hi-C showed compact diagonal heterochromatin loops without TADs, which are drastically different from common interphase Hi-C maps (the majority of unsynchronized cell ensemble are in interphase), continuous bridging of intermediate cell-cycle phases is urgently needed. The global restructuring of chromosomal architecture during the progression from mitosis into G1 phase was revealed by in situ Hi-C study in mouse cells, which is illustrated in Ref. [10]. In principle, one could take such “snapshots” data as the constraints to simulate chromatin dynamics by computational means (e.g., [11]); however, without intermediate ground truth, such simulated (augmented) structures cannot be reliable because there are infinitely many possible structures that can perfectly fit such limited ensemble constraints. Because of the high density and fast speed, detailed mitotic chromosome dynamics has been the most difficult to study and is the focus of new advanced technology development.

Firstly, how a mitotic self-entangled state is converted into an unentangled interphase state during mitotic exit has been studied by Hi-C, polymer simulations, and multi-contact 3C [12]; regarding the dual function of Topoisomerase-II, disentanglement during mitotic exit involves regulated topo II activity, and decondensation drives topo II-dependent decatenation of mitotic loops (Figure 3).

Secondly, the genome folding dynamics are controlled by different forms of condensins (condensin-I, condensin-II) and cohesins (cohesive-cohesins and extrusive-cohesins), made of different combination of structural maintenance of chromosome (SMC) complexes in an orderly fashion [13]; many detailed results have been obtained by a combination of cell biology, imaging, proteomics, Hi-C, and polymer modeling, in which various combinations of SMC complexes were acutely depleted before synchronous entry into mitosis [14]. It was found that condensins I and II extrude chromatin in loops with a speed of about 2 kb per second in living chicken cells. Through polymer modeling and by testing results of simulations against experimental imaging and Hi-C data, three “rules of engagement” were identified that dictate what occurs when these fast-moving complexes encounter one another along the chromatin fiber, which are visualized in graphical abstract of Ref. [14]. First, when a condensin runs into an extruding cohesin complex, cohesin is removed from the chromatin, and condensin continues its loop extrusion, thus “erasing” interphase organization. In the absence of condensin, this organization persists well into mitosis even though the chromosomes condense into amorphous structures. Second, when a condensin runs into a cohesive cohesin complex, it steps over it and keeps extruding. As a result, sister chromatids can separate

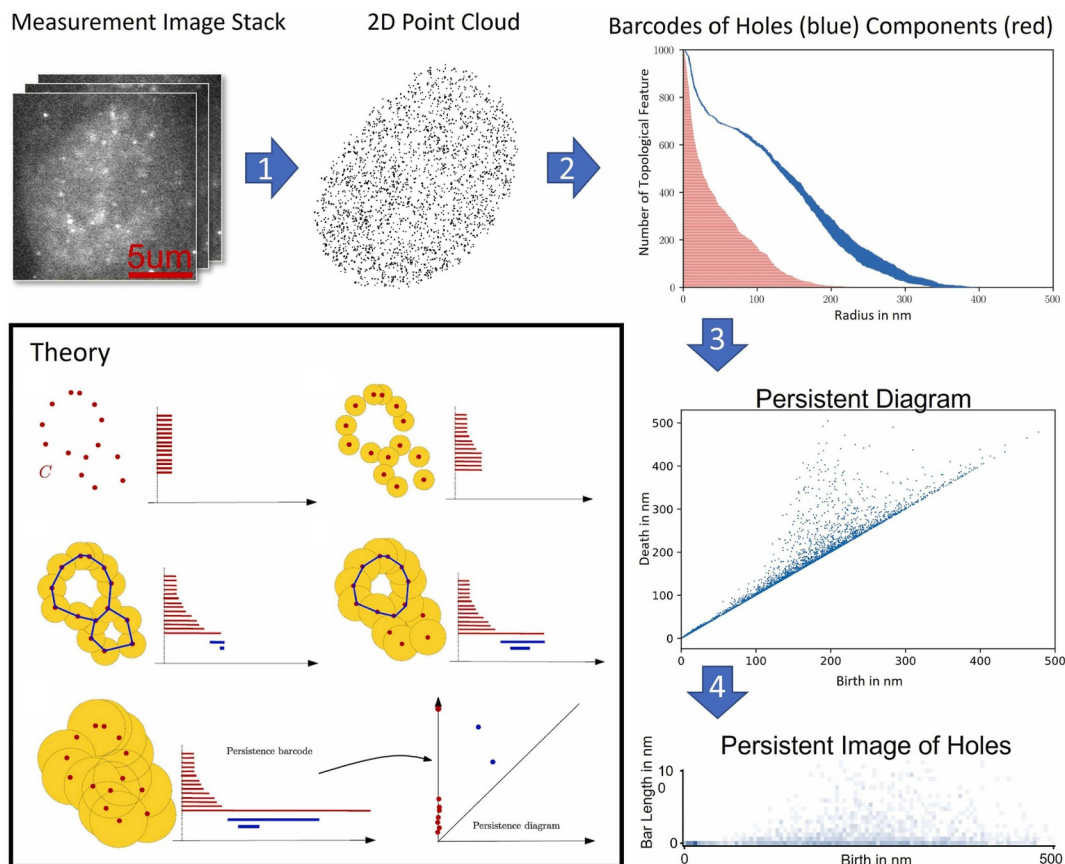


FIGURE 2 Topological persistent analysis [9].

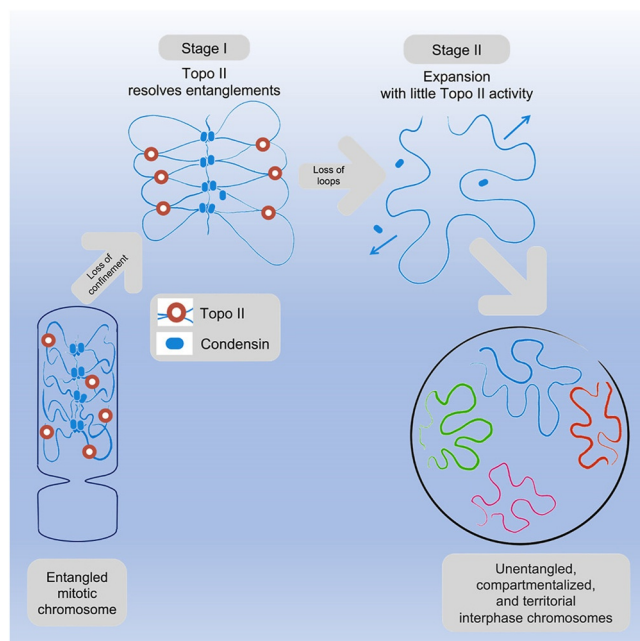


FIGURE 3 Topoisomerase-II-dependent two-stage exit model from mitosis [12].

their axes, while remaining cohered between the tips of their loops. Third, when a condensin approaches another condensin, it stalls. This produces arrays of consecutive

loops. Despite these collective efforts, it is not yet fully understood how the actions of the two condensin complexes are mechanistically differentiated and spatio-temporally coordinated during the progression of mitotic chromosome assembly; the roles of histones [15] and nuclear F-actins [16] are still missing in the picture. Additionally, pioneer transcription factors, such as FoxA1, could remain bound to mitotic chromosome as “bookmarks” so that their target genes would be rapidly reactive during mitotic exit, as is presented in Refs. [17, 18].

A more subtle and controversial question is whether the majority of loops in interphase are really formed by extrusion in vivo, as an alternative to the DNA extrusion model; in contrast, the DNA–DNA capture model has already been proposed to be the unified model for cohesin function in both sister chromatid cohesion and chromatin loop formation [19]. The truth may lie in both, some loops are initially produced by extrusion (e.g., growing symmetric anti-45° diagonal signatures), whereas others are initially produced by DNA capture. There are several models; for example, the dimeric inchworm model [20] has been proposed to further explain continuous extruding mechanism, high-resolution structures of key cohesin intermediates in vivo are needed to validate, refute, or modify these models. On the other hand, one should not simply

ignore in vitro models, as in biology, anything that could happen under some conditions. The key is to what extent, and which in vitro result actually has functional consequences.

After mitosis, nuclear envelope has to be reassembled back (Figure 4 and Ref. [21]). Chromatin DNA needs to dynamically interact with the new lamina (LAM) and the nuclear pore complex, and later to interact or associate with various NBs. Most of these dynamic details are currently either fragmented or missing, awaiting for more multi-spatial and multi-time-scale data and integrative modeling. However, high-resolution mapping of their genomic interactions dynamically in vivo remains challenging due to transient interactions and the potential loss of rare components during data integration. CRISPR-GO was the first life-cell imaging technology that was able to mediate rapid de novo formation of CajB at desired chromatin loci, and caused significant repression of endogenous gene expression over long distances (30–600 kb) [22]. Although this and similar technology hold great promise for 4D genome mapping, their adoption is currently limited by technical hurdles, practical challenges in research settings, and the need for further development and refinement.

4 | STRESS-RESPONSE DYNAMICS

Stress conditions such as heat shock, starvation, microbial infection, UV light, chemical insult, DNA damage, senescence, and oncogene activation often result in

reorganization of chromatin architecture transiently or permanently at different levels of the hierarchy. Most common stress responses are reversible because the genetic regulation network is designed to maintain cellular homeostasis (e.g., chromatin conformation remains stable upon extensive transcriptional changes driven by heat shock, Ref. [23]), but if cellular or DNA damage are beyond repairable, more drastic genetic defense mechanisms such as cellular senescence (permanently cell cycle arrest) or apoptosis (programmed death) will be activated. Senescence-associated heterochromatin foci (SAHF) are regulated through pathways such as cGAS-STING, which link cytoplasmic DNA sensing to chromatin remodeling—a vivid illustration may be found in ref. [24]. Two common types of senescence (RS = replicative or OIS = oncogene induced) cell nucleus states are well studied. Both types display accumulation of senescence-associated beta-galactosidase (SA- β -gal), activation of Rb/p16 and p53/p21 pathways, morphological changes, and induction of the senescence-associated secretory phenotype, which is clearly seen in nuclear staining. However, early studies in OIS and RS have suggested that TADs and compartments change in a subtle or even controversial way, until multi-omics and careful DNA-FISH finally began to reveal their difference and connection [25]. They identified senescence-associated heterochromatin domains (SAHDs) and showed that the differential intra-versus inter-SAHD interactions (increase distal B-B compartments) lead to the formation of SAHF (SAHFs,

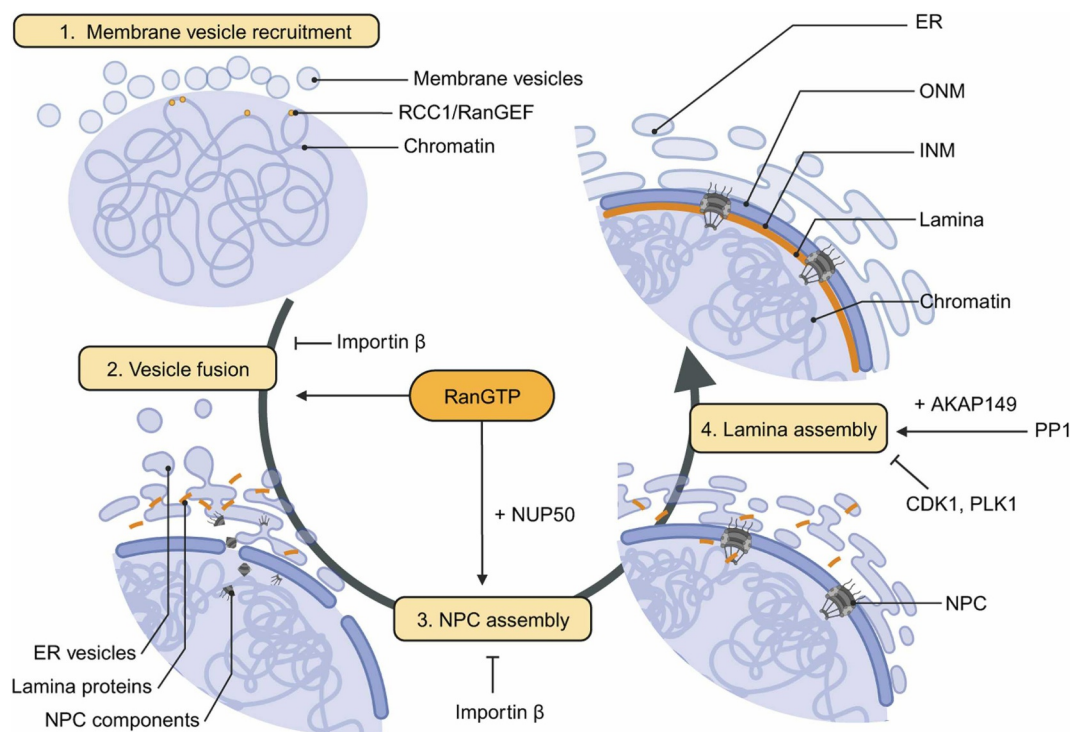


FIGURE 4 Four-step nuclear envelope reassembly (NER) [21].

senescence specifically delocalized LAD cores) in OIS but not in RS. This OIS-specific configuration brings active genes located in genomic regions adjacent to SAHDs in close spatial proximity and favors their expression. This study also identified DNMT1 as a factor that induces SAHFs by promoting HMGA2 expression and, upon DNMT1 depletion, OIS cells transition to RS-like cells. Given that Alu and L1 densities differ in cytogenetic bands, researchers [26] have examined their distribution after X-chromosome inactivation linked to Barr body (BB) or the formation of senescent-associated heterochromatin foci (SAHFs). Alu-rich regions remain outside both SAHFs and BB, affirming that the BB is not the whole chromosome but a condensed, L1-rich core. Hi-C analysis of senescent cells demonstrates large (around 10 Mb) G-bands remodel as a contiguous unit, gaining distal intrachromosomal interactions as syntenic G-bands coalesce into SAHFs. Striking peaks of Alu within R-bands strongly resist condensation. Because liquid–liquid phase separation is one of the key mechanisms of forming condensates regulating chromatin dynamics and gene expression, localizing chromatin associated architectural RNAs (arcRNAs) [27] together with RRI sequencing technology [28] is very valuable for understanding the function of associated 3D genome structures, bridging different chromatin scales to cytogenetic scales.

Other poorly understood states or processes, such as post-mitotic quiescence, contact inhibition, chromoplexy/chromothripsis, or polyploid coenocyte formation, present unique challenges for future 4D genome research. Furthermore, the plasticity of dynamic attractor states, iPS-reprogramming, trans-differentiation, horizontal gene transfer, *etc.* pose additional complexity in integrating genome function with structure.

5 | EVO-DEVO LANDSCAPE AND ATTRACTOR PLASTICITY

Although cells are the fundamental unit, heritable traits are selected at the individual organism level (through development) and genes are optimized, fixed, or evolved at population level through selection. To distinguishing different cell types (through bifurcative differentiation or convergent organogenesis), their lineage relations must be traced back to their origins (e.g., progenitor cells, blastocyst or fertilized eggs) in order to uncover the process through which they are actually formed dynamically. To construct the ultimate map from genes to phenotype/behavior (e.g., conservation and changes of 4D genome in different cell types to different populations), comparative and backwards coalescence modeling with multi-scale, multi-modality spatial omics and imaging data will be necessary.

During development, cells traverse a landscape of regulatory states—conceptualized as Waddington’s

epigenetic landscape—where chromatin conformation plays a pivotal role. Chromatin loops and enhancer–promoter interactions change dynamically during various developmental processes such as zygotic genome activation (ZGA), gastrulation, and organogenesis. Figure 5 [29] shows the differentiation of retinal cell types from pluripotent stem cells (before day 60) to more mature retinal cells (at day 200). These stages of development are shown in conjunction with progressive changes to chromatin’s 3D organization (red, white, and pink maps on right).

E. Lewis’s paper “A gene complex controlling segmentation in *Drosophila*” [30] had a huge impact on our way of thinking about development and evolution and was recognized by the Nobel Prize in 1995, opening the door to a molecular genetic analysis of development. Meanwhile the landmark results of the embryonic lethal screen carried out in *Drosophila* [31] had started to decipher the genetic control of early embryogenesis in animal models. Soon four clusters in mammals and two half-clusters in flies suggested that the hypothesized ancestral cluster already implemented this peculiar colinear expression strategy, “Evo-Devo” reflects these long-lasting interactions between ontogeny and phylogeny. Interestingly, so called “Hox clock,” yet it may rather be considered as a “Hox timer” in Figure 6 [32] due to its non-recursive structure.

The bottleneck illustrates the developmental period where embryos are most similar to one another, with Haeckel’s original drawings copied into the magnifying glass [33]. The neck of the hourglass corresponds to the coordinated implementation of two time-regulated mechanisms: the segmentation clock (an in-trans oscillation) and the Hox timer (an in-cis progression). This associates a genetic progression in cis with a “morphological oscillation” in-trans in Figure 7 [32].

Recent work has highlighted how transcription factors such as MyoD and CTCF contribute to lineage-specific chromatin reorganization [34]. The CAPTURE-3C-seq method was developed to study how the locus control region is controlling the human β -globin developmental switch [35]. Moreover, enhancer hijacking events, such as those found in T-ALL (HOXA13), illustrate how disruptions in 3D genome organization lead to disease phenotypes [36]. The evolutionary conservation of chromatin structures (C-scaffolds) suggests deep regulatory logic shared across species [37].

Emergence of “fountains” and boundaries after ZGA, as shown in Figure 8 [38], are highly conserved in animals, and such structure disappeared after cohesin KO (indicated by “dRad21”). This model predicts that such extruding constitute starting points of chromosome folding during early development, likely serving as sites of targeted cohesin loading; extrusion fountains may be the hallmarks of chromosome organization emerging upon the ZGA transition. These cohesion-dependent “excursion fountains” (they are a transient or

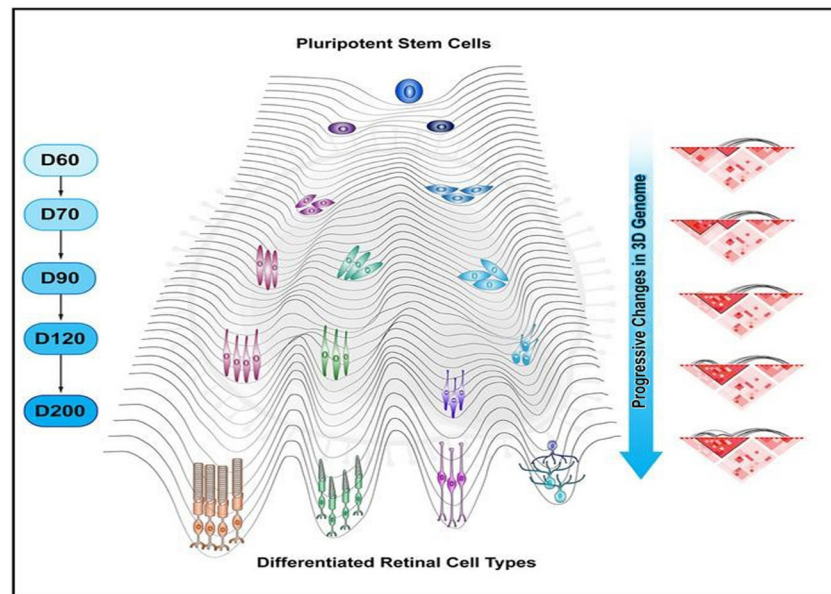


FIGURE 5 Waddington's epigenetic landscape in retinal cell differentiation [29].

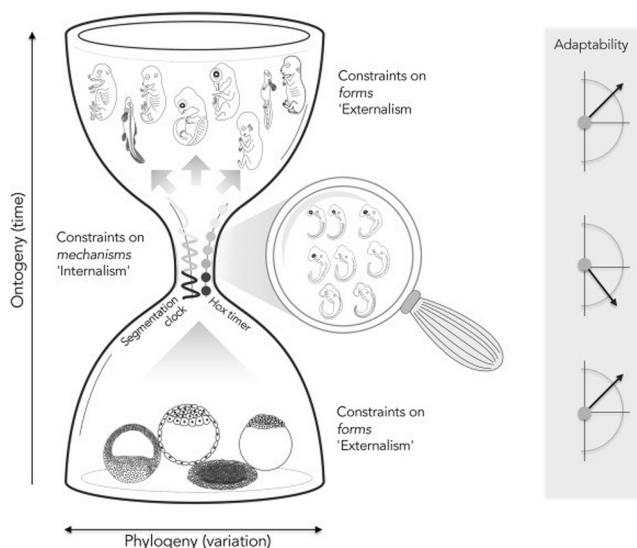


FIGURE 6 Developmental hourglass [32].

specialized state of looping that lacks the stable boundaries provided by CTCF) should not be confused with DNA “replication fountains” (or “Jets” during DNA elongation).

On the other hand, comparative 3D genome analysis of macaque fetal brain with human and rodent reveals evolutionary innovations during primate corticogenesis, which is outlined in the graphical abstract of Ref. [39]. The human-specific loops are significantly enriched in enhancer–enhancer interactions, and the regulated genes show human-specific expression changes in the subplate, a transient zone of the developing brain critical

for neural circuit formation and plasticity. Both conserved or evolved 3D genome organization models require further in vivo developmental validations, in order to functionally linking them to transcriptional regulation networks.

I have mentioned the relationship between 4D genome organization and DNA replication in the context of mitotic cell cycle above, but replication origin specification and configuration vary substantially between eukaryotic species and in some cases co-evolved with gene-silencing mechanisms. It was proposed that centromeres and origins of DNA replication were originally derived from a common element and later separated during evolution, which is presented graphically in Ref. [40]. In the G1 phase, origins of replication are “licensed” by assembling pre-RCs, which include ORC, Cdc6, Cdt1, and MCM proteins. In some organisms such as *Drosophila* and *Xenopus*, there's strong evidence suggesting that ORC (and the pre-RC) nucleates cohesin binding to DNA. However, other research studies suggest that cohesin and MCM are loaded onto chromatin independently in humans, but interaction between cohesin and MCMs can involve pausing, suggesting that MCMs can act as active barriers with binding sites, potentially through interaction motifs like the YDF motif found in human MCM3. The ORC2 subunit has been shown to be involved in regulating chromatin loops by repressing CTCF binding at certain sites, which could indirectly affect cohesin function. It would be very important to resolve different reports and give a decisive validation of the proposed evolutionary model.

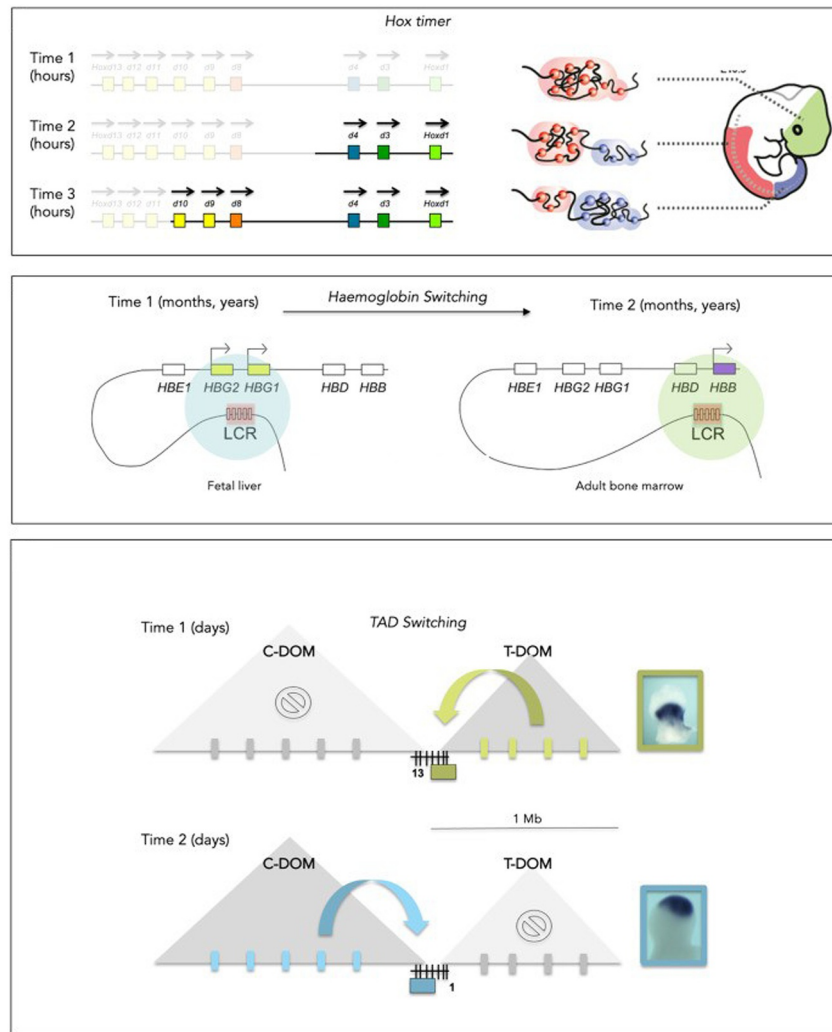


FIGURE 7 Various time processes in multigenic regulations [32].

6 | EMERGING TECHNOLOGIES AND FUTURE DIRECTIONS

Recent technological advances are beginning to overcome several longstanding barriers in 4D genome research, particularly in resolving chromatin dynamics across spatial, temporal, and functional dimensions. These tools not only offer improved resolution and throughput but also enable perturbative and predictive approaches to test the causal architecture of genome organization.

6.1 | Integrative single cell multi-modality spatial profiling and live imaging

Multiplexed spatial transcriptomics and imaging techniques, such as ORCA, Hi-M, MERFISH, seqFISH+, DNA-PAINT, and OligoSTORM, now allow the localization of hundreds of RNA species or genomic loci within

single cells [41]. Combined with CRISPR-based tagging platforms such as CasDrop and CRISPRainbow, researchers can track the motion of specific loci over time and across cell states [42]. Together with more advanced single cell or multi-modality sequencing technologies, such as Micro-C & Hi-C3.0, SPRITE, GAM, Spatial-ATAC-seq, Dip-C [43], HiRES [44], ChAIR [45], researchers are beginning to reveal context-dependent chromatin movements and rare interactions that were previously inaccessible to bulk or static assays.

6.2 | Single-molecule and real-time modalities

Long-read technologies such as nanopore and SMRT sequencing enable direct detection of DNA modifications (e.g., 5 mC, 6 mA) and higher-order chromatin features without amplification artifacts. Time-resolved adaptations of these platforms—such as pulse-chase

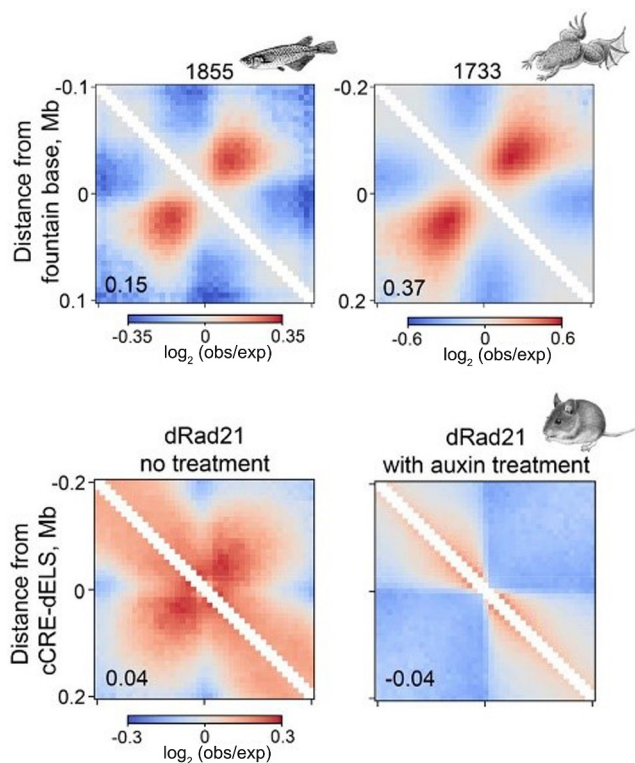


FIGURE 8 Emergence of fountains and boundaries after zygotic genome activation (ZGA) [38].

labeling with 5-ethynyluridine (EU) or metabolic labeling of nascent chromatin—offer windows into the kinetics of genome architecture during processes, such as mitosis, DNA damage repair, or ZGA [46]. Recently, live-cell imaging & CRISPR-Tagging (utilizing dCas9-fluorescent tags to track the real-time movement of specific loci in living cells, allowing researchers to observe loop formation and extrusion as it happens), time-resolved DamID & Repli-seq (track how chromatin-lamina interactions or replication timing programs change over hours or through the cell cycle) and single-cell 4D Maps (like SPIN or FLAMINGO that can combine sequencing and imaging data to reconstruct thousands of single-cell 3D structures at resolutions as high as 1 kb.) are adding 4th dimensional information (temporal dynamics).

6.3 | Perturbative tools and synthetic genomics

A new class of inducible perturbation systems—such as optogenetic dimerizers (e.g., CRY2/CIB1) or degron-based protein depletion—enable spatial and temporal control over chromatin regulators like CTCF, cohesin, or Polycomb [47]. These tools allow researchers to test the sufficiency and necessity of chromatin loops or compartments in shaping transcriptional states. Moreover,

synthetic genomics approaches, including artificial chromosomes or rewired enhancer–promoter networks, offer testbeds to dissect minimal rules of nuclear organization.

6.4 | Computational modeling and predictive frameworks

Polymer-based simulations and agent-based models continue to elucidate the emergence of chromatin domains from molecular interactions [48]. Concurrently, machine learning models trained on multi-omic data—such as Akita and Enformer—now predict chromatin conformation, accessibility, and gene expression from DNA sequence alone (Ref. [49], taking into account the epigenome caveats mentioned above). These approaches are increasingly used to prioritize regulatory variants in disease-associated loci and to infer latent regulatory grammars. It is intriguing that recent predictive modeling [50] showed that ChromEM-resolved chromatin packing domains (PDs; 50–200 nm) are not TADs (200–500 nm).

6.5 | Toward unified models of the 4D genome

Emerging integration frameworks, such as MOFA+, totalVI, and scTriangulate, aim to unify genome structure, epigenetic modifications, transcription, and protein localization in a coherent model [51]. These multi-modal datasets, particularly when collected from synchronized or perturbed systems, may finally enable causal inference in chromatin dynamics and resolve debates around hierarchy versus plasticity in nuclear organization. In addition to 4D Genome Browser (4DGB) & 4DGB Workflow, AlphaGenome, and Automated Recommendation Tool, computational foundation modeling is certainly a new area of active development (e.g., *Evo2HiC*, [52]). More emerging tools, such as c.Origami & FLAMINGO (AI-driven frameworks that predict cell-type-specific 3D folding directly from DNA sequence and accessibility, facilitating in silico genetic screens to see how mutations alter 4D structure) and VACmap ([53], a new non-linear long-read aligner that improves the detection of complex structural variants that disrupt 3D genome architecture), are expected to accelerate 4D genome understanding.

Despite these advances, challenges remain in harmonizing data types, resolving rare states, and modeling feedback between genome topology and function. Nonetheless, these technologies set the stage for predictive and re-programmable 4D genome biology.

7 | CONCLUSION AND OUTLOOK

The field of 4D genomics stands at an inflection point. New tools have revealed previously hidden layers of genome architecture, yet fundamental challenges persist in synthesizing these findings into a coherent picture. Chief among these are the issues of scale integration, causal directionality during dynamic transitions, and the evolutionary logic of chromatin reorganization.

To address these bottlenecks, the field must prioritize multi-modal integration, improved spatial and temporal resolution, and cross-species comparisons. Techniques such as scHi-C, CAPTURE-3C, multiplexed imaging, and machine learning-based modeling of chromatin dynamics will be essential. Only through such integrative and iterative approaches can we resolve the pitfalls and limitations in our current understanding and chart a path toward predictive 4D genome biology.

AUTHOR CONTRIBUTIONS

Michael Zhang: Conceptualization; formal analysis; writing—original draft; writing—review and editing.

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Michael Q. Zhang is an Editorial Board Member of *Quantitative Biology*, who was excluded from the peer-review process and all editorial decisions related to the acceptance and publication of this article. Peer-review was handled independently by the other editors to minimize bias.

DATA AVAILABILITY STATEMENT

This review article does not generate any new data.

ETHICS STATEMENT

There is no ethics statement that should be disclosed in this study.

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