



Attach and stretch: Emerging roles for genome–lamina contacts in shaping the 3D genome

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Abstract

A large proportion of the metazoan genome is spatially segregated at the nuclear periphery through genomic contacts with the nuclear lamina, a thin meshwork of lamin filaments that lines the inner-nuclear membrane. Lamina-associated domains are believed to contribute to the regulation of gene transcription and to provide structural three-dimensional support to the organization of the genome in A and B compartments and topologically associating domains. In this review, we will evaluate recent work addressing the role of lamina-associated domains in three-dimensional genome organization and propose experimental frameworks that may expand our understanding of their interdependence.

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Keywords

3D genome organization, Lamina-associated domains, Chromatin compartments, Topologically associating domains.

Introduction

Transcriptional activity is tightly controlled through a coordinated regulation at different genomic layers. A detailed comprehension of the interconnectivity between these layers of regulation is key in deciphering how transcriptional networks are established and maintained. In this review, we focus on 3D genome organization with an emphasis on the relationship between spatial genome positioning, compartmentalization, and topology. More specifically, we will focus on the role of genome scaffolding at the nuclear lamina (NL) in

shaping the three-dimensional (3D) genome in topologically associating domains (TADs) and active (A) and inactive (B) chromatin compartments. Finally, we propose experimental frameworks to start untangling these relationships in single-cell settings.

Lamina-associated domain organization and function

Lamina-associated domains (LADs) in mammalian systems are broad (median size ~ 0.5 Mb) genomic regions that show low transcriptional activity [1,2]. LADs correspond to the perinuclear, dense heterochromatin seen by electron microscopy [3]. In genomic studies, LADs are commonly identified by DNA adenine methyltransferase identification or DamID [4,5]. They are characterized by low gene densities, high A/T content, late replication timing, and an enrichment for long interspersed nuclear element [1,6,7]. Besides mammals, LADs also exist in *Drosophila melanogaster* and *Caenorhabditis elegans* with broadly similar characteristics [8,9]. Across species and cell types, LADs are associated with H3K9me2/3 [1,10–12] and to a lesser extent H3K27me3 [1,12,13]. H3K9me2/3 is involved in the establishment and maintenance of genome–NL contacts [3,11,14,15], and, in *C. elegans*, the protein CEC-4 has been identified as an anchor between this methylation mark and the NL [16]. Up to date, such an anchor protein has not been identified in mammals.

LADs can be subdivided into regions that associate with the NL in all cell types (constitutive, cLADs) and regions that facultatively associate with the NL in a cell type–specific manner (facultative, fLADs). Facultative LADs are enriched for H3K27me3 and harbor genes that are developmentally regulated [6,12,17]. Constitutive LADs are enriched for H3K9me2/3, depleted of genes and contain more A/T sequences [6,12,17]. Because cLADs are NL-associated across cell types they are hypothesized to provide a structural 3D suspension framework [6]. Interestingly, in zygotes [18] and mouse embryonic stem cells (mESCs) [6] a high proportion of all LADs constitutes cLADs [6,18]. Thus, this structural backbone may represent a default state of LAD organization in totipotent (zygote) and pluripotent (mESC) cells that is then overruled by lineage-specific transcriptional programs [2,6].

In all species and cell types, gene activities in LADs are low, suggestive of a role in transcriptional regulation. Indeed, random integrations of thousands of reporters in LADs resulted in a marked attenuation of transcriptional activities [18]. Vice versa, the integration of LAD-embedded promoters in non-LAD genomic environments resulted in elevated transcription levels [19]. These results show that LADs represent gene-repressive environments; however, ~10% of genes is able to escape transcriptional repression despite embedding in LAD–chromatin [19]. While gene repositioning toward the NL generally results in transcriptional silencing, LADs are not necessarily directly involved in the mechanisms of repression. Gene repositioning toward the nuclear interior is not always associated with gene activation [2,20–22], and, conversely, gene silencing may precede repositioning of loci toward the NL [23]. For example, during neuroblast differentiation in *Drosophila*, the *hunchback* gene repositions towards the NL only after transcriptional repression. Thus, NL repositioning is not required for gene repression. Yet, prevention of *hunchback* association with the NL by lamin depletion results in a delay in cellular commitment and a prolonged neuroblast competence window [23]. In line with this example, the overall emerging role for the NL appears to involve restricting or potentiating lineage-specific gene activities through sequestering or releasing genomic regions from the perinuclear NL compartment [16,21–25]. Thus, instead of a role in direct gene silencing, the association of genomic loci with the NL may reinforce transcriptional states.

Relationship between LADs, TADs, and chromatin compartments

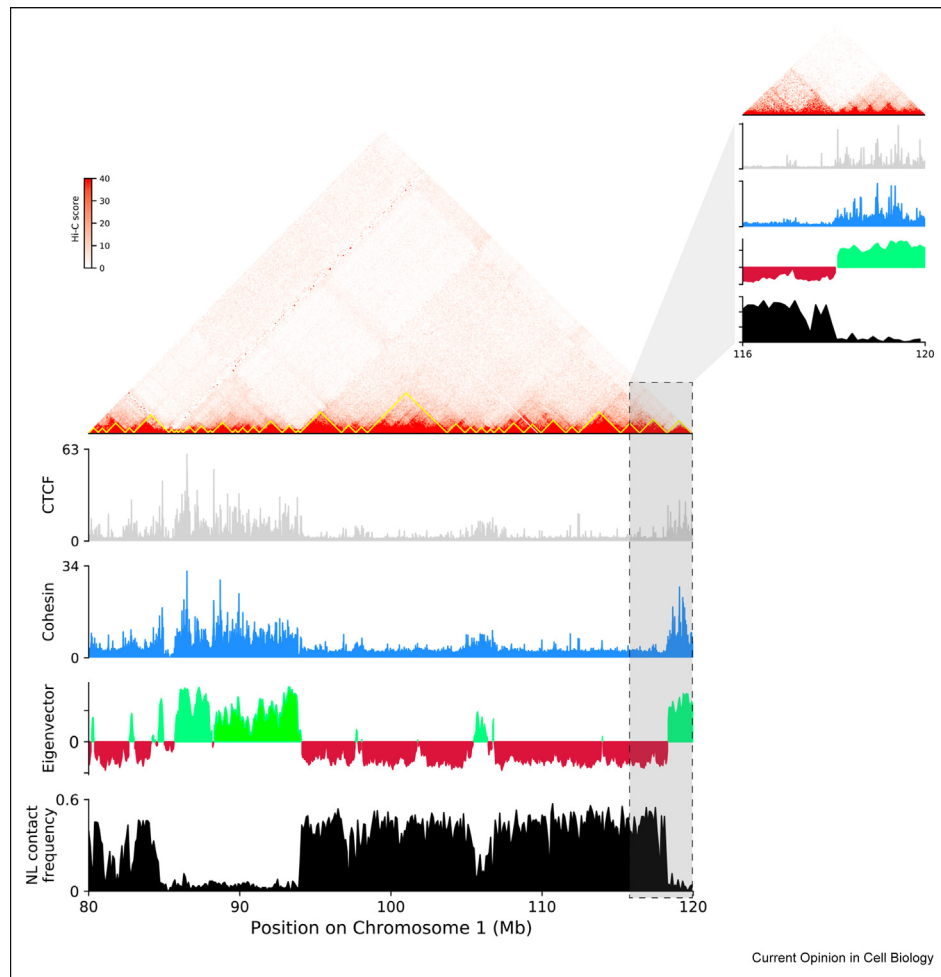
The technological advancements in chromosome conformation capture (3C) methods over the past decade have provided detailed molecular information into genome folding. At a mega-base scale, the genome is partitioned into A and B compartments [26]. At a lower genomic scale, chromosomes are organized into 0.1–1 Mb TADs, which represent genomic regions with preferential intradomain contacts while restricting interactions with neighboring domains [27,28]. LADs correspond to transcriptionally repressive B compartments [7,17,28,29] (Figure 1), and sharp boundaries corresponding to LAD-to-inter-LAD (iLAD) transitions generally coincide with TAD boundaries [28–30] (Figure 1). In addition, LAD borders are demarcated by CTCF binding sites similarly to TADs [1] (Figure 1). LAD and TAD borders do not perfectly overlap; yet this may be at least in part related to differences in data quality, bin size, thresholds, and algorithms used to individually define TADs and LADs [30].

Thus, LADs share common features with TADs and compartments, but what is the interconnectivity

between spatial genome positioning and genome compartmentalization and topology? Recent work in *C. elegans* embryos lacking the NL-anchoring protein CEC-4 [16] suggests that perinuclear attachment of chromosomes helps segregate the genome in active and inactive chromatin types [31,32]. In addition, perinuclear chromatin anchoring by CEC-4 contributes to the partitioning between heterochromatin domains at the opposite chromosomes arms [31,32]. Removal of CEC-4 also results in a global increase in inter-TAD interactions and genome compaction, which is presumably due to a loss of NL anchoring—enforced chromosome stretching [31,32]. On the contrary, lamin depletions in mESCs and *D. melanogaster* S2 cells result in a decompaction of NL-associated chromatin [33,34]. This difference may be partially explained by the difference in the biological system (cultured cells versus whole organisms), or more likely by the difference in the extent of loss of perinuclear anchoring between CEC-4 (severe effect) and lamin depletions (mild effect). Nevertheless, a common denominator between all studies is that a loss or reduced genome–NL anchoring results in blurring of genome segmentation into distinct active and inactive compartments [31–34].

The increase in contacts between the distal heterochromatic chromosome arms in the absence of CEC-4 suggests that, in the absence of NL anchoring, the self-organizing properties of H3K9me2/3 chromatin drive the sequestration of B compartments [35–37]. Indeed, removal of the major enzymes responsible for H3K9 methylation resulted in a reduction in associations between the distal arms [32]. This suggests that anchoring of chromosomes to the NL may also promote partitioning of the heterochromatic chromosomal arms that are otherwise inclined to self-associate. In nocturnal mammals, rod cells of the retina naturally lack chromosome anchoring because of the combined absence of Lamin-B receptor (LBR) and lamin A/C [38], which makes the rod cells an excellent system to study and disentangle the contribution of 1) chromosome perinuclear anchoring and 2) self-organization of matching chromatin states on genome compartmentalization [39]. In rod cells, heterochromatin is packed together into a single cluster in the nuclear interior and euchromatin is positioned at the nuclear periphery. Recent Hi-C experiments in rod cells, combined with polymer simulations, revealed that the self-organizing properties of the B compartments are indeed the prime driving force in establishing genome compartmentalization in these cells [40]. Taken together, these data suggest that LADs may not directly contribute to the genome compartmentalization in A and B compartments *per se*, yet may be essential for the proper spatial partitioning of B compartments and the resulting stretching of chromosomes to achieve optimal segregation of active and inactive chromatin domains [31–34].

Figure 1



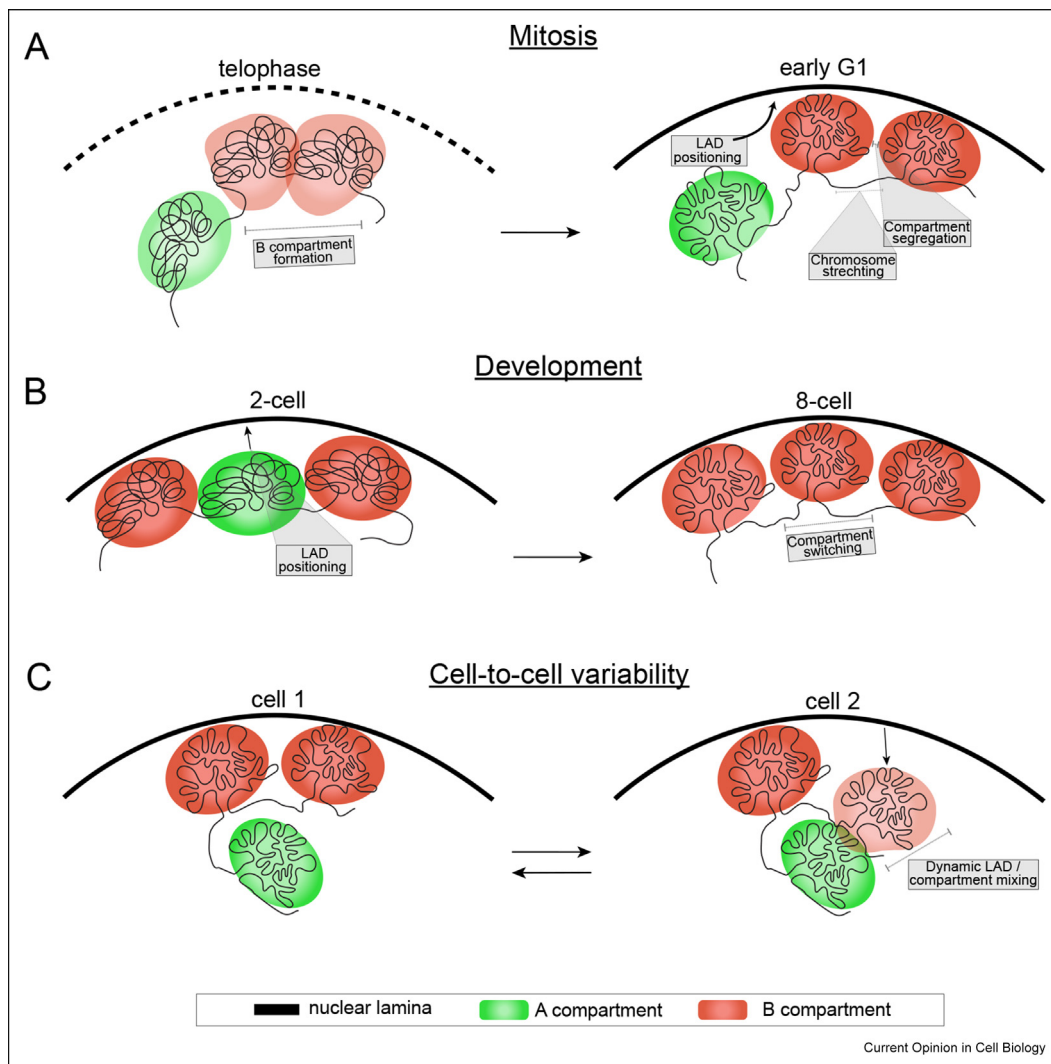
The overlap between LADs, compartments and TADs. NL association (black) generally coincides with compartment switching (green: A, red: B compartment). In addition, LAD borders may at least partially overlap with CTCF (gray) and cohesin (blue) bound TAD borders (yellow), an example of which is highlighted here. Data shown is from mouse embryonic stem cells: Hi-C [61], CTCF ChIP-seq [61], cohesin (SMC1) ChIP-seq [62], and LmnB1 scDam&T [56]. LADs, lamina-associated domains; NL, nuclear lamina; TADs, topologically associating domains.

Next, to further our understanding of the hierarchies and interdependencies between multiple modes of 3D genome organization, it is essential to study changes in LADs and genome topological organization in dynamic settings such as in a development and/or cell-cycle context. In cycling cells, LADs and the topological genome organization both are re-established shortly after mitosis [3,41–43]. In mouse preimplantation development, LADs were previously shown to be established *de novo* in the mouse zygote, before zygotic genome activation, the establishment of TADs, and the consolidation of compartments [18,44,45]. Thus, both the cell cycle and preimplantation development offer ideal frameworks to study the sequence of events and the interdependencies between the reformation of LADs, genome compartmentalization and topology (Figure 2 and discussed below).

Rebuilding the 3D genome organization in preimplantation embryos

Before the fusion of the paternal and maternal genomes, typical LADs can be observed in the paternal pronucleus, while LADs in the maternal pronucleus appear less defined [18]. An exceptional ~70% of the LADs in the paternal pronuclei constitute cLADs. Thus, in the absence of transcription, the genome may be organized into a basic spatial suspension framework mainly comprised of cLADs. Then, during the first cleavage stages, and after transcriptional activation, the spatial genome organization is dramatically reorganized [18]. Intriguingly, while generally NL repositioning is accompanied by concordant compartment switching from A to B compartments at the same cleavage stage, for ~11% of the genome, localization toward the NL at the two-cell stage does not coincide with but precedes

Figure 2



The dynamic interplay between spatial genome positioning, compartmentalization, and topology. (A) To enable mitosis, the cell's interphase nuclear architecture is completely rearranged. The telophase marks the onset of re-establishing the interphase 3D genome, commencing with the aggregation of intrachromosomal LADs into single large B compartment separated from the iLADs [43,51]. Upon mitotic exit, the nuclear envelope reforms allowing this B compartment to interact with the NL, causing its segregation into multiple smaller domains [3,51]. (B) During mouse preimplantation development, genome–lamina interactions precede the switching from A to B compartment for a remarkable ~11% of the genome [18]. In addition, TADs are not detected until the eight-cell stage, whereas genome–lamina interactions and compartments are defined as early as the two-cell stage [18,45]. (C) Finally, genome–lamina interactions have been shown to be subject to high cell-to-cell variability [3,17], which may cause genomic compartments to intermingle [58]. 3D, three-dimensional; LADs, lamina-associated domains; NL, nuclear lamina; TADs, topologically associating domains.

compartment switching from A to B at later stages [18] (Figure 2). Collectively, these findings suggest that NL positioning may help pioneer changes in genome compartmentalization in early mouse development [18]. What the exact relationship is between spatial genome repositioning and compartment switching requires further investigations and the development of new powerful single-cell multi-omic and multiplexed DNA Fluorescence In Situ Hybridization (FISH) approaches may help unravel such interdependencies (Figure 2 and see below).

Re-building 3D genome organization after mitosis

During interphase, LADs are confined to a <1 micron-sized layer lining the NL [3,46]. In the prophase of mitosis, the nuclear envelope breaks down, and, consequently, the contacts between the genome and NL are lost. How and when do LADs re-establish in the daughter cells? Several recent studies have now provided new spatiotemporal insights into LAD reformation after mitosis. Previously, through the use of a method to illuminate and live trace the genomic

interactions with the NL, it was shown that LADs are spatially redistributed in daughter cells [47]. At least a fraction of the LADs positioned at the NL before mitosis are associated with the nucleolus as nucleolus-associated domains [3,48–50]. Interestingly, the same genomic tracing strategy combined with multiplexed DNA FISH in mouse cells revealed that, before nuclear envelope reformation in the telophase, LADs on the same chromosomes aggregate into a single compartment, separated from iLADs. Then, such aggregates partition into multiple smaller domains upon NL association [3,51]. This suggests that genome compartmentalization occurs independently of NL tethering and precedes spatial repositioning of genomic loci towards the NL. Indeed, B compartments were found to first appear in the telophase and consolidate during early G1 [43] coincidentally with the spatial segregation and repositioning of the genome towards the NL [42]. Thus, similarly to rod cells, genome compartmentalization may initially be driven by B–B associations [40] resulting in large domains encompassing entire chromosomes. Then, analogous to the stretching in *C. elegans* [31], the initiation of genome–NL contacts in the early G1 phase may help partition B compartments to further facilitate genome segregation into defined A and B compartments [31–34].

Repositioning of genomic regions to the NL appears orchestrated through H3K9me2-mediated anchoring: a mark that is inherited through mitosis and can thus serve to ‘bookmark’ LADs for re-association [14,15]. Intriguingly, the development and implementation of a better time-resolved version of DamID recently showed that immediately after mitosis genome–NL contacts are most prominent at the distal ~25 Mb chromosome arms as opposed to the middle of chromosomes, a pattern that is reversed as interphase progresses [52]. In addition, previous microscopy studies have already postulated that telomere anchoring may contribute to reformation of chromatin NL contacts [53]. Thus, reminiscent to the mechanism in *C. elegans* [31], genome anchoring of the distal ends of chromosomes may represent a more universal mechanism required to ‘stretch’ chromosomes to partition and re-establish genome organization in defined territories of similar activities.

Cell-to-cell heterogeneity in LAD organization

DamID has recently been optimized to profile genome-wide protein–DNA interactions in single cells. In agreement with microscopy observations, single-cell lamin B1 contact maps reveal extensive heterogeneity in spatial genome positioning [17]. Constitutive LADs display the most invariable contacts with the NL, again confirming the potential role for these regions as a structural 3D backbone. The LADs with more variable

contacts correspond to fLADs and thus contain genes that will be activated in a cell type–specific manner [17]. If the role of LADs is indeed to ‘lock-in’ transcriptional states (see earlier), cell-to-cell variability of genes in fLADs may provide a window of opportunity for transcription factors to target these developmental genes when timing is correct. Because in each individual cell different ‘sets’ of fLADs are localized toward the nucleoplasm, cell-to-cell heterogeneity in LAD organization may provide a stochastic framework for the acquisition of lineage-specific transcriptional programs. Transcriptional activation and the associated changes in chromatin organization would then likely ensure confinement of such regions to the nucleoplasm over consecutive cell divisions [54]. It would be interesting to test this model in early developmental settings when the first major cell fate decisions are established [55].

The recent development of multimodal single-cell sequencing and imaging approaches now offer new opportunities to study cellular transcriptional dynamics in light of the underlying functional genomic context. With scDam&T [56,57] transcriptional status can be obtained in combination with LAD profiles in the same cell. A recent method reports imaging of chromatin structure and genomic positioning together with transcriptional activities [58]. With the latter, genomic contact maps can be inferred from spatial proximity measurements, and, therefore, this method is highly powerful in unveiling the structural principles of genome compartmentalization associated with the variations in spatial genome positioning [31,58]. With such approaches, spatial arrangements of A and B loci were found to show extensive variability in degrees of segregation (or mixing) [31,58]. It would be very interesting to relate such variations to spatial genome positioning and implement this method to carefully dissect the interdependencies between genome–NL reformation and genome 3D folding in cells exiting mitosis. However, unlike scDam&T, multiplexed FISH is not (yet) a genome-wide approach. Therefore, future combinations between scDamID [17] and scHiC [59] or scSPRITE [60] could be very valuable to uncover genome-wide associations between LADs and compartments in diverse biological settings.

Conclusions

Genome–lamina interactions appear to have a key role in the correct formation of the 3D genome. Dissociation of LADs from the NL results in intermingling of otherwise separated activate and inactive genomic compartments, as well as increased interactions between neighboring TADs. However, we are only beginning to understand the interdependencies of these multiple modes of 3D genome organization. For example, it remains elusive whether forced detachment of cLADs would impede chromatin architecture. Or

conversely, how does the genome interact with the NL in the absence of 3D chromatin topology? Dynamic reorganizations of the 3D genome, such as during development and the cell cycle, may provide unique opportunities to better disentangle how the multiple modes of genome organization are interrelated. In addition, future multi-omic single-cell readouts of spatial genome positioning with genome compartments and topology combined with selective perturbation experiments may greatly aid in such discoveries.

Credit author statement

Pim Rullens: Writing – Reviewing and Editing, Conceptualization, Data analysis, Jop Kind: Writing – Original Draft, Conceptualization, Supervision

Conflict of interest statement

Nothing declared.

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