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Chromosome organization in 4D: insights from *C. elegans* development

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Eukaryotic genome organization is ordered and multilayered, from the nucleosome to chromosomal scales. These layers are not static during development, but are remodeled over time and between tissues. Thus, animal model studies with high spatiotemporal resolution are necessary to understand the various forms and functions of genome organization *in vivo*. In *C. elegans*, sequencing- and imaging-based advances have provided insight on how histone modifications, regulatory elements, and large-scale chromosome conformations are established and changed. Recent observations include unexpected physiological roles for topologically associating domains, different roles for the nuclear lamina at different chromatin scales, cell-type-specific enhancer and promoter regulatory grammars, and prevalent compartment variability in early development. Here, we summarize these and other recent findings in *C. elegans*, and suggest future avenues of research to enrich our *in vivo* knowledge of the forms and functions of nuclear organization.

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Introduction

Almost a century ago, nuclear material was characterized as either visibly compact heterochromatin or loosely-packed euchromatin [1], but in the last 20 years, diverse 'flavors' of chromatin and chromosome conformations have been identified. We have learned that chromosomes occupy distinct territories within nuclei, composed of large compartments and smaller topologically associating domains (TADs), features that are conserved in many organisms [2–4]. Many conceptual advances were fueled by new and improved sequencing-based or imaging-based technologies (Figure 1); [5,6], which are continuously uncovering emergent properties of genome folding across species.

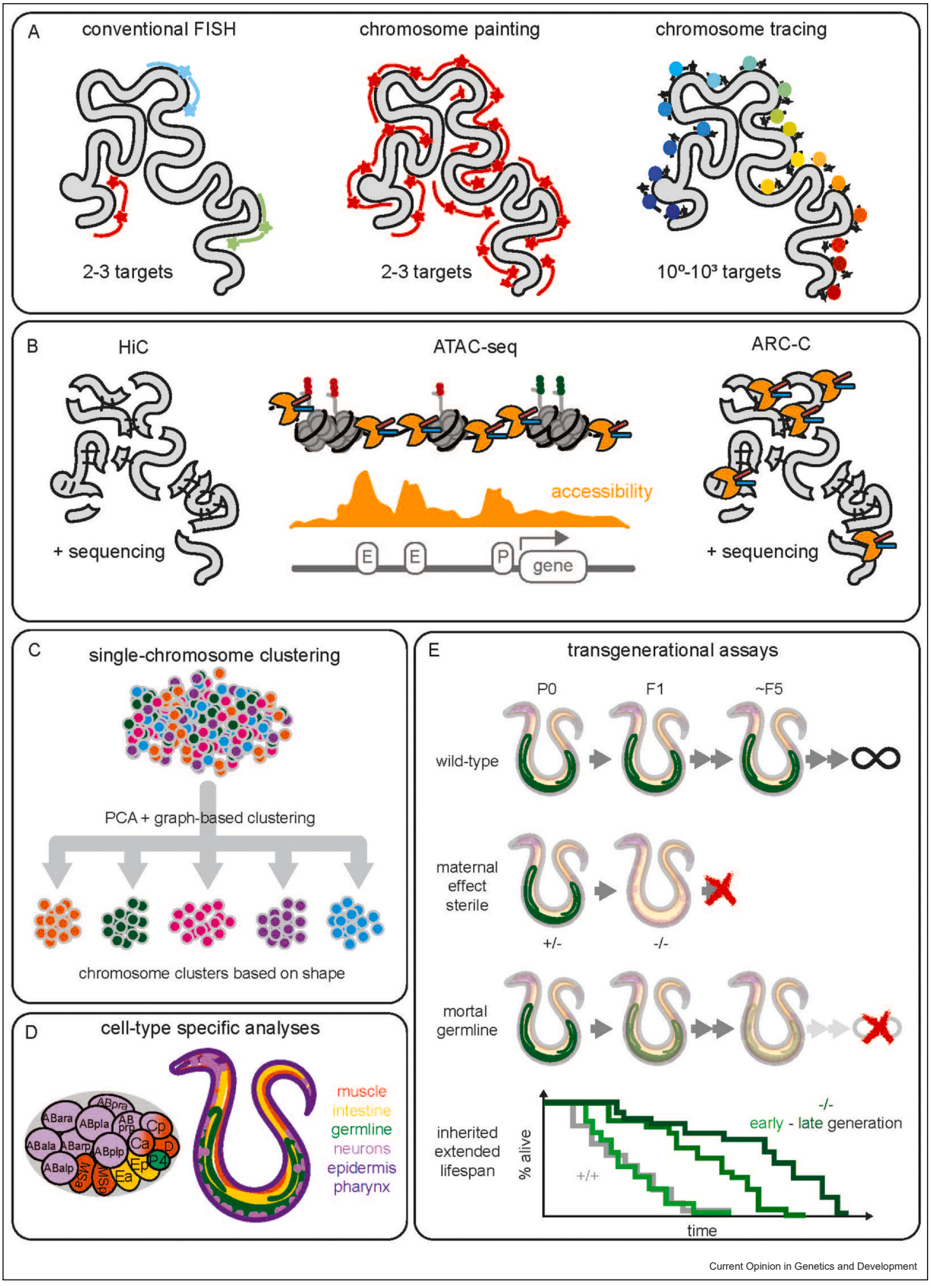
Lamina association and epigenetic remodeling have emerged as important regulators of genome folding. Histone modifications function in diverse biological processes including inter/transgenerational memory [7], cell fate and fertility [8–14], and lifespan [15]. Here we focus on the roles of histone modifications and the lamina on the 3D organization of the *C. elegans* genome and recent advances that address its function or regulation. *C. elegans* presents a powerful system to study nuclear organization in an animal throughout development, aging, and across generations. It is well suited to genetic, cytological, biochemical, genomic, and phenotypic approaches, both at the single-cell and the population level (Figure 1). These attributes currently enable researchers to build upon the already rich knowledge of the linear genome sequence, histone modifications [16–18] and fully mapped cell lineage [19,20] to study 4D genome folding.

We highlight recent progress in the study of genome folding in *C. elegans* from the local (topologically associating domains), to chromosomal (compartments, territories) scales throughout development, periodically contrasting with data from other species. We present these recent findings, current challenges, and potential avenues for future research on how the organization of the genome relates to its function.

X-chromosome topologically associating domains promote longevity and stress responses but not dosage compensation

TADs are contiguous regions of self-interacting DNA that sequester genes with their regulatory elements in 3D space, and are thought to play a role in regulating gene expression [2]. Genome-wide chromosome conformation capture (Hi-C; Figure 1) studies have mapped robust TADs on the *C. elegans* X chromosome [21–24] (Figure 2), and their boundaries appear stably positioned throughout development [25]. The majority of TAD boundaries on the X are established at *rex* sites (*r*ecruitment *e*lements on *X*), which are bound by the condensin-containing dosage compensation complex

Figure 1


A
conventional FISH
chromosome painting
chromosome tracing
2-3 targets
2-3 targets
10⁰-10³ targets
B
HiC
ATAC-seq
ARC-C
+ sequencing
accessibility
E
E
P
gene
+ sequencing
C
single-chromosome clustering
PCA + graph-based clustering
chromosome clusters based on shape
D
cell-type specific analyses
ABpra
ABara
ABpla
AB prp
Cp
ABala
ABarp
ABplp
Ca
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ABalp
MSpp
MSap
Ea
Ep
P4
muscle
intestine
germline
neurons
epidermis
pharynx
E
transgenerational assays
P0
F1
~F5
wild-type
∞
maternal effect sterile
+/-
-/-
mortal germline
inherited extended lifespan
% alive
+/+
-/-
early - late generation
time
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Experimental approaches to study the forms and functions of nuclear organization in *C. elegans*. **(a)** . FISH-based methods. (left) Conventional FISH. Fluorescently-labeled oligonucleotides complementary to few genomic targets determine their locations *in situ*. Typically the number of targets per experiment is limited by the illumination source to 1–3 (middle) Chromosome painting. A large library of fluorescently-labeled oligonucleotides which coat entire chromosomes (or large portions of chromosomes) determine their size and positions [58]. Typically the number of targets per experiment is limited by the illumination source to 1–3 (right) Chromosome tracing. Chromosome tracing uses sequential fluorescently-labeled oligonucleotide hybridizations to probe the locations of up to thousands of targets with super-resolution [32,78]. All FISH-based methods are inherently single-cell, and can be used to directly measure chromosome volumes, proximity between loci, and proximity to other nuclear elements (e.g. nucleoli, lamina, speckles). **(b)**. Sequencing-based methods. (left) Hi-C. Hi-C measures the frequency of ligation between fragments of DNA genome-wide, through cross-linking of nearby sequences, digestion with restriction enzymes, ligation and sequencing. Contact frequency is then used to infer chromosome conformation [21–24]. (middle) ATAC-seq. ATAC-seq measures genome accessibility to a transposase which simultaneously inserts sequencing adapters into open regions. The number of reads after sequencing for a particular element (e.g. enhancer/promoter) therefore correlates with its accessibility [38,39,79,80]. (right) Accessible Region Conformation Capture (ARC-C). Akin to combining Hi-C with ATAC-seq, ARC-C measures interactions between regulatory elements genome-wide. DNA undergoes fixation, limited DNase I digestion, ligation and transposase tagmentation followed by sequencing [25]. To date, published Hi-C, ATAC-seq, and ARC-C analyses in *C. elegans* have been population-based. **(c)**. Single-chromosome clustering. Single-chromosome clustering classifies and quantifies sub-populations of chromosome conformations from a heterogeneous pool in an unbiased manner. Using chromosome tracing (or single-cell Hi-C) data, individual chromosomes are separated in high-dimensional PCA space based on pairwise distances between internal points, and similar chromosomes are grouped by graph-based clustering [32]. **(d)**. Cell-type-specific analyses. The complete cell lineage of *C. elegans* has been mapped [19,20], thus every cell has a name and a known history and fate. This knowledge enables researchers to perform cell-type and cell lineage-specific analyses at any point in organism development. Schematics of a 16-cell embryo with cell names and an adult are shown, with cell types color-coded to show the neurons, germline, muscle, intestine, epidermis, and pharynx. **(e)**. Transgenerational assays. The fertility and lifespan of *C. elegans* strains can be measured for many generations in a relatively short time span. Fertility is used as a sensitive readout of proper germline development and function, and several chromatin factors have shown maternal effect sterile (sterility after 1 generation) or mortal germline (progressive sterility) phenotypes [10,11]. Lifespan of animals in many generations can be measured to determine if any accumulated shortening or extension occurs [15]. (+/+) wild-type, (+/-) heterozygote, (-/-) mutant.

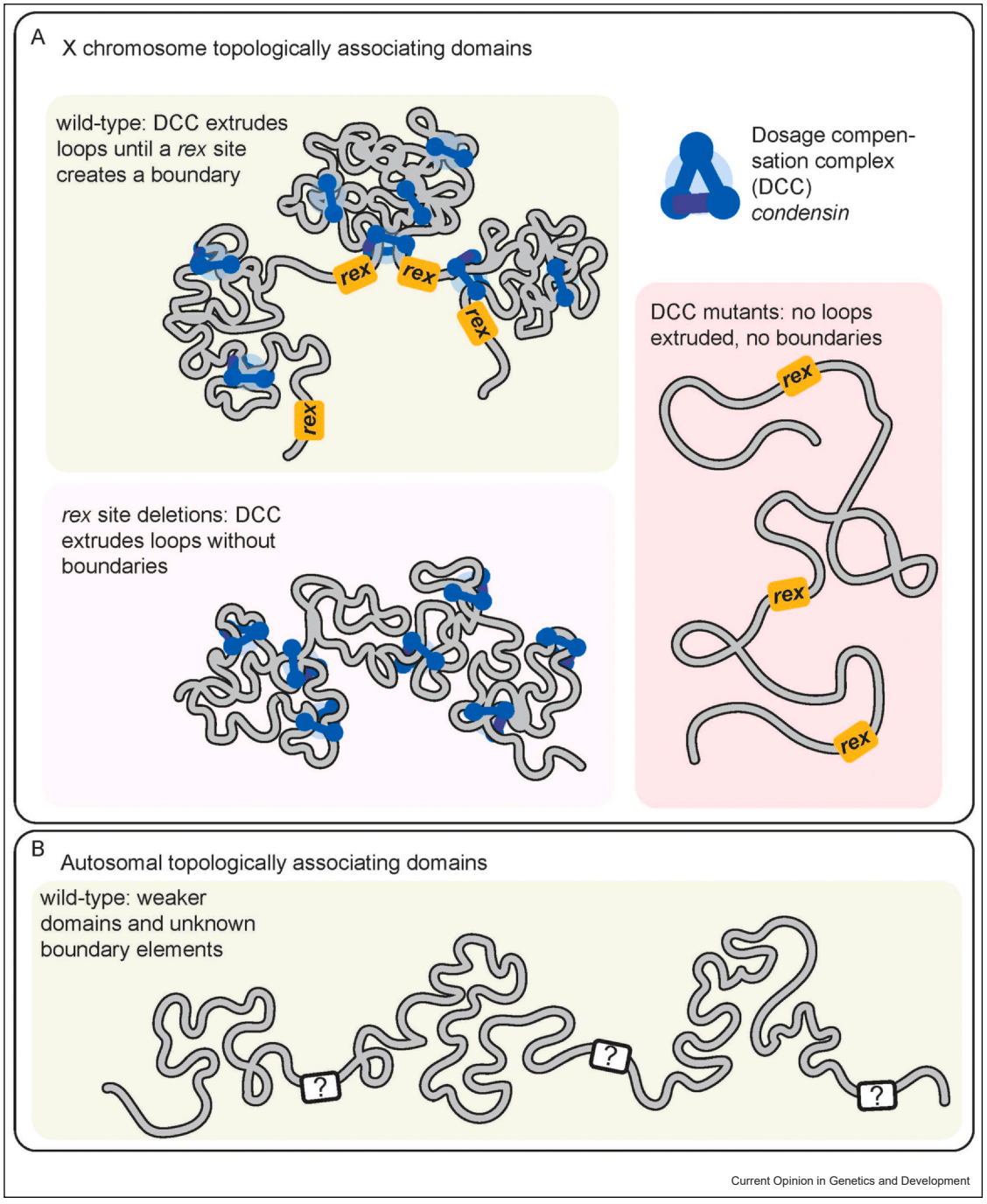
(DCC) (Figure 2). Mutation of *rex* sites or the DCC eliminates X-linked TAD boundaries, and insertion of a *rex* site in ectopic genomic locations is sufficient to form a new boundary [22,26]. The DCC is a type of X-chromosome-specific, structural maintenance of chromosomes (SMC) complex, with an associated histone H4 lysine 20me2 demethylase (DPY-21) and DNA binding proteins [27]. Studies with other species have proposed that SMC complexes establish TADs by extruding loops of chromatin through ring-like structures in an Adenosine Triphosphate (ATP)-dependent manner, and the DCC likely functions similarly [22,24,28].

The DCC contributes to TADs in a second way. DCC localization at strong *rex* sites generates TAD boundaries in the absence of other known boundary proteins in *C. elegans* [28]: DCC complexes may act as architectural proteins to establish a boundary when present in multicopy at high-occupancy *rex* sites. In addition to the DCC, H3 lysine 9 methylation (H3K9me) promotes both boundary strength and interactions between boundary regions, perhaps by promoting chromosome compaction and affinity [23].

Apart from its role in TAD formation, the major function of the DCC is to equalize gene expression along the X chromosome between the sexes (XO males and XX hermaphrodites). It does so by downregulation of genes on each hermaphrodite X by 50% [27]. Mutation of the DCC leads to a two-fold increase in X-linked gene expression due to increased loading of RNA polymerase II at promoters [27]. Surprisingly, the TAD boundary-forming role of the DCC is not involved in the dosage compensation process. When *rex* sites along the entire X chromosome are deleted, their associated boundaries are eliminated, but transcriptional downregulation of the entire X chromosome still occurs [22]. While previous studies with other species examined the role of TADs through genome-wide loss of SMC proteins or CCCTC-binding factor (CTCF) (which may have pleiotropic effects), the precise disruption of boundary establishment across the entire *C. elegans* X chromosome enabled the elegant dissection of the DCC's dual roles [22,28]. Genes located adjacent to the deletions are expressed at wild-type levels in embryos [22]. However, these X-linked TAD boundaries are important *after* embryogenesis, and their loss results in accelerated ageing and reduced thermotolerance in adult animals through an as yet unknown mechanism [22]. Curiously, premature aging is restricted to hermaphrodites, not males, suggesting that while delayed, this effect could be due to disrupted dosage compensation. This study, therefore, demonstrated that a temporal disconnect can exist between when a particular domain forms and when its insulation from other genomic loci is phenotypically important for diverse biological processes [22].

The surprising inconsequentiality of TAD boundaries in dosage compensation raised the question of how transcriptional downregulation is achieved. How does the DCC prevent polymerase loading? The differences between *rex* mutants and DCC mutants provided key insights into the transcriptional downregulation mechanism (Figure 2). In addition to establishing TAD boundaries at *rex* sites, the DCC promotes local chromatin interactions at the 0.1–1 Mb scale [22], likely by its SMC loop extrusion function throughout the X chromosome [24]. In one study, the dosage compensated

Figure 2



Topologically associating domains in *C. elegans*. **(a)** . X-chromosome TADs are built by the DCC, which binds rex sites and compacts chromatin, likely by loop extrusion. rex sites are the boundary elements of TADs, but are not necessary for chromatin compaction by the DCC. Without the DCC, the X chromosome loses boundaries and compaction [21,22,24]. **(b)**. Autosomal TADs are less compact than X-chromosome TADs. Both their boundary elements and molecular drivers are currently unknown [21].

structure was proposed to resemble a rosette of loops [24]. In addition, the associated DPY-21 demethylase represses gene expression, compacts chromatin, and modulates DCC dynamics [29,30]. These features are not disrupted by *rex* mutations, suggesting that they are the likely elements needed for dosage compensation.

Autosomal topologically associating domains: domains of variable size and unclear origins

While the X chromosome has the most pronounced ~1 Mb TADs, TADs also exist on the autosomes [21,25,31,32](Figure 2). Like TADs in other species, these TADs are contiguous stretches of the chromosome, underscoring their TAD-like affiliation; however, they appear weaker than TADs in other species [21]. There may be multiple reasons for this: *C. elegans* autosomes do not recruit the DCC, and lack known strong boundary elements like *rex* or CTCF sites. Additionally, the diversity of cell types and stages of cells used in previous studies may have diluted any cell-specific boundaries beyond the detection limit. A recent analysis showed a good correlation between TAD boundaries and changes in gene expression in worms and other animals [31]. Boundaries appeared at the transition zones between active and inactive genes along the genome. Therefore, transcriptional activity may contribute to TAD boundaries on the autosomes, or TADs may sequester regions with distinct transcriptional states. Given the diversity of expression patterns that co-exist in all stages of *C. elegans* development [33,34](Figure 1), it would not be surprising if transcription-associated TAD boundaries were blurred when averaged over millions of cells, as has been observed in mammalian systems [35].

In addition to TADs of canonical sizes throughout the genome described above (~1 Mb), *C. elegans* possesses smaller TAD-like structures that are less than 200 kb and often under 20Kb [25]. These miniTADs may reflect the small size of *C. elegans* genes and genome, and clustering of ~3 genes within a TAD-like domain in multiple species [25,31] (Table 1). Genes and their cis-regulatory sites in *C. elegans* are often under 10 kb [16] and are packed tightly along the chromosome or within co-transcribed operons. Because of their small size, the miniTADs were not detectable by previous HiC or chromosome tracing, but were identified by a new method called ARC-C [25] (Figure 1). ARC-C resembles high throughput chromosome conformation capture (Hi-C), but enriches for interactions between regulatory regions by using DNase and transposase-mediated tagmentation [25]. This method also revealed that genes with similar expression patterns often co-localize both within and between miniTADs, and that transcription factors may promote these interactions [25]. For example, the zinc finger B Lymphocyte-induced Maturation Protein-1 homolog (BLMP-1) promotes physical interactions between its actively transcribing target genes [25]. Active genes bound by BLMP-1 failed to cluster in *blmp-1* mutants, indicating that transcriptional activation may depend on BLMP-1-induced physical clustering. Alternatively, BLMP-1 target genes may depend on transcription to generate clusters. Intriguingly, miniTADs are also characterized by their enrichment for active or inactive chromatin states, and miniTADs of similar state have enriched compartment-like interactions (see below) by ARC-C similar to recent observations in other species [25,31] (Table 1). The relationship between the kb-scale miniTADs and Mb-scale TADs has not yet been elucidated, but it is possible that Mb TADs are built by compartment-like interactions between neighboring miniTADs.

Compartment boundaries and compositions are variable in early embryogenesis

Compartments are large 3D associations of euchromatic or heterochromatic TADs from noncontiguous regions along a chromosome [36]. *C. elegans* autosomes carry B 'inactive' compartment chromatin, enriched for silencing histone modifications such as di or tri methylated histone H3 lysine 9 (H3K9me2/me3), H3K27me3, and lamina association along their arms. The centers of autosomes comprise a large gene-rich, repeat-poor A 'active' compartment, with gradually tapering borders between compartment classes [17,21,37] (Table 1). Thus, the typical *C. elegans* autosome has a linear B-A-B composition along its arm-center-arm. This compartmentalization differs from that of vertebrates, where multiple A and B domains span one chromosome. The difference may reflect the smaller size of the chromosomes (≤20 Mb vs 50–250 Mb).

One distinction between *C. elegans* large central A compartments and those of other organisms is that active histone modifications are not enriched in the centers, but spread throughout the chromosomes. This lack of correlation between active modifications and the large A compartment may be due to the averaging of many distinct cell types and mixed embryo stages in the population chromatin immunoprecipitation - sequencing (ChIP-seq) and HiC experiments, thus different chromatin signatures [38,39] would be diluted. Studies from other organisms predicted autosomes would form a horseshoe conformation in 3D, with the B regions clustered together in space, and this is observed in post-gastrulation *C. elegans* embryos [32,40]. A Compartments typically interact with A compartments from other chromosomes [23]. The X chromosome has a single clear B-like compartment on the left arm, and the remainder resembles A compartment chromatin [17,21,37].

In addition to chromosome-wide configurations, the miniTADs also cluster together at a finer scale. Active miniTADs and regions marked by H3K27me3 are both

Table 1

Classes of chromosome conformations in *C. elegans*.

Name	Genomic size	Features	Known Regulators	Techniques of detection & Stage/ tissue	Similar conformations in other species
Territory	13.8–21 Mb	entire chromosome	MES-3 DCC (chrX) Nuclear Argonautes (chrX,[82])	chromosome painting (single molecule, *adult germline, oocytes, neurons, intestine, epidermis*)	Territory
B Compartment (Autosome arms)	~5.5 Mb	Enriched for constitutive heterochromatin H3K9me2/3 and H3K27me3 modifications, repetitive sequences, lamina association, lower gene density	MET-2, SET-25 for boundary position CEC-4 for arm-arm interaction and boundary position	Hi-C (population, *mixed embryo*) chromosome tracing (single molecule, *early embryo*) ARC-C (population, *L3*)	B compartment
A Compartment (Autosome centers)	~5.6 Mb	Higher gene density, low lamina association, less repetitive elements, A-A inter-chromosomal interactions *not enriched for activity-associated modifications as in other species	MET-2, SET-25 for boundary position	Hi-C (population, *mixed embryo*) chromosome tracing (single molecule, *early embryo*) ARC-C (population, *L3*)	A compartment
TAD	~1 Mb	~200 genes, strong boundaries on chrX and weaker on autosomes *unknown boundary elements on autosomes	DCC (chrX)	Hi-C (population, *mixed embryo*) chromosome tracing (single molecule, *early embryo*) ARC-C (population, *L3*)	Mammalian low-resolution HiC domains[83], TAD-like or single-cell domains by FISH[84]
miniTAD (autosomes)	~20 kb Alternating all along the length of the autosomes	~3 genes Active = broadly and germline active genes, marked by H3K36me3 and other modifications associated with gene activity Inactive = regulated genes, marked by H3K27me3 interactions between miniTADs of same type form fine-scale compartments	MET-2, SET-25 promote interactions between inactive miniTADs Presumably PRC and H3K36 methyl transferase and transcription?	ARC-C aggregate analysis (population, *L3*) ChIP of histone modifications (population, *mixed embryo*)	compartmental domains from high-resolution HiC *(Drosophila KC167 cells, mammalian cultured cells)*[31], budding yeast domains from micro-C[85], nucleosome clutches by imaging (*mammalian cultured cells*)[86]
lamina associated domain (**LAD**)	~ 4 Mb on each autosome arm and chrX left arm comprising many punctuated ~58 kb domains and ~12 kb gaps	Discontinuous sections in chromosome arms, overlapping with B compartment chromatin (population) Enriched for satellite repeats, helitrons, and inactive genes (population) Variable association all along chrV (single molecule)	CEC-4 BAF-1 LINC complex MET-2, SET-25	ChIP (population, *mixed embryos*) DamID (population, *muscle, intestine*) Chromosome tracing (single molecule, *early embryo*)	LAD
Enhancer-Promoter interactions	1 Kb–1 Mb	Germline and somatic accessibility signatures	Transcription factors and chromatin regulators	ATAC-seq to identify accessible regulatory elements (population, *L4-young adult sorted germline, intestine, muscle, epidermis, neurons*)[38,39,80] ARC-C to identify interactions between regulatory elements (population, *L3*)	Enhancer-Promoter interactions *larger genomic distances in other species

A comparison of chromosome conformations detected to date in *C. elegans*, their features, genetic determinants, and similarities to conformations detected in other species

enriched in contacts with similar chromatin state [25]. The enrichment is relatively weak, with less than a two-fold effect as detected by chromosome capture methods for *C. elegans* and also for other animals, which may reflect a weak affinity between distant chromosome domains. Alternatively, chromosomes are highly dynamic in cells, which can disrupt TAD and compartmental interactions. For example, SMC proteins extrude chromatin into loops [28], different regions of chromosomes attach and detach from the nuclear lamina [41], and they also move dynamically in 3D [35,42,43].

Until recently, compartment characterization in any organism was based on averages from thousands or millions of cells, but recent single-chromosome data suggest that the chromosome conformations of individual cells do not resemble the population average. Embryonic chromosomes have a wide range of morphologies [44–49], and this is also true for *C. elegans*, which exhibit variable compartment boundaries and chromosome folding patterns [32]. Two tools are needed to understand chromosome variability: i) single cell or ideally, single chromosome data and ii) computational approaches to identify and quantify conformational trends.

Chromosome tracing combined with single-chromosome clustering is a powerful means to analyze variability [32] (Figure 1). These approaches have revealed that only a minority of chromosomes in early embryos have a canonical horseshoe conformation of clustered arms, as had been predicted by Hi-C. The remaining conformational pools resemble lopsided barbells, with arm regions far apart in nuclear space. These different clusters of chromosomes have unique compartment boundary positions, which are sharper and stronger than the apparent boundary of the population average [32]. Clustering analysis also identified highly variable regions that drive different conformations, and uncovered invariable regions that are common to most chromosome conformations [32]. These data indicate that neither compartments nor their boundaries are fixed. Extensive single-cell variability was recently seen in other organisms at the TAD level [35], supporting the idea that a number of prevalent conformations can coexist along a range of organizational sizes, from the smaller TADs to larger compartments. This variability raises the question to what degree histone modifications vary between chromosomes. Compartments are thought to reflect epigenetic status [31], suggesting histone modifications across broad regions of the genome may be highly dynamic between individual cells or chromosomes.

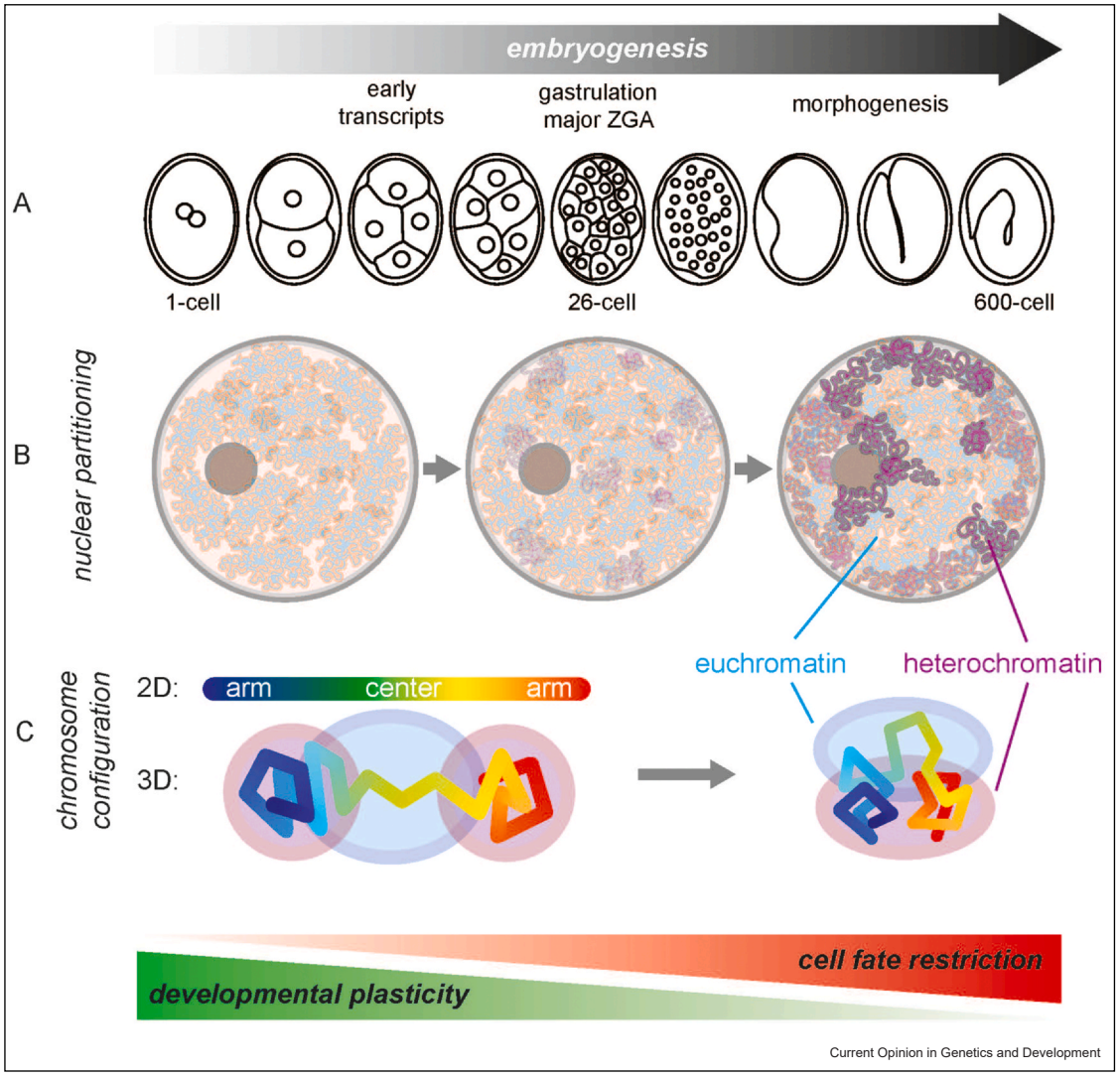
Compartments arise *de novo* during embryonic development

A diversity of whole-chromosome conformations is observed during early embryogenesis (see above). Despite this variability, even before canonical B compartments arise during gastrulation, the ensemble folding pattern of the chromosome arms is distinct from that of the nascent A compartment. The arms exhibit long-range internal folding, over many Mb, and are consequently more compact, compared to the A compartment [32]. At gastrulation, the arms begin to consolidate into a single B compartment, thereby creating a horseshoe shape that is consistent with the Hi-C data for older, more differentiated embryos [21–23,32](Figure 3). A similar gradual emergence of compartments during embryogenesis has also been observed for mice, flies, and frogs during zygotic genome activation and gastrulation [44–49], raising the question of whether these species also have extended barbell-like configurations at early stages, for stretches of nascent B-A-B domains.

How is the timing of compartmentalization achieved? While the drivers of complete A/B compartmentalization have not yet been elucidated, recent studies have shown that constitutive heterochromatin (enriched in the B compartment) is driven by histone methylation. H3K9me, particularly H3K9me2 and H3K9me3, accumulates gradually during embryogenesis and is required to form constitutive heterochromatin *de novo* [50,51] (Figure 3). histone methyltransferase like (MET-2), a SET Domain Bifurcated Histone Lysine Methyltransferase (SETDB1)-family methyltransferase which mediates H3K9me1/2, is a maternally deposited protein that is present before fertilization, so the presence of MET-2 *per se* cannot explain the acquisition of H3K9me. Instead, the slow accumulation of H3K9me is due to the gradual nuclear enrichment of MET-2, which generate puncta within nuclei [50,52]. MET-2, in turn, is regulated by its binding partners, the synMuv B protein LIN-65, which induces nuclear accumulation of MET-2, and ADP Ribosylation factor Like GTPase 14 Effector protein homolog (ARLE-14), the orthologue of ARL14 Effector Protein, which promotes association of MET-2 with chromatin [50,53]. MET-2-generated H3K9me1/2 is a likely substrate for SET-25-derived H3K9me3 and heterochromatin [4,51]. SET-25 also produces H3K9me3 by a *met-2*-independent pathway that relies on small RNAs, the nuclear RNAi pathway and the MBT domain protein abnormal cell lineage (LIN-61), which together target SET-25 to transposons and repeats [54]. It is not yet known if this latter pathway is developmentally regulated, or if it plays a role in nuclear puncta formation or large-scale chromosome conformation.

H3K9me3 provides a binding platform for HP1-like proteins HPL-1/2 [55], chromodomain proteins [56] and LIN-61 [54]. *In vitro* studies suggests that HP1 can oligomerize, which promotes the formation of polymer phase-separated states and presumably B compartments [36]. Data from *C. elegans* have shown that H3K9me promotes proper A/B compartmentalization [23,25]. Loss of H3K9me weakens both HPL-2/HP1 association with

Figure 3



The gradual emergence of genome compartmentalization. **(a)**. Schematic of embryonic development in *C. elegans*. Early zygotic transcripts are first detected in the 4-cell embryo. At ~26-cells, gastrulation (movement of cells into the embryo interior) begins, coinciding with the major burst of zygotic genome activation (ZGA). These milestones are followed by morphogenesis and elongation into a worm-like shape [81]. **(b)**. Schematic of nuclear partitioning into visibly densely-packed heterochromatin and diffuse euchromatin. These classes are best visualized by electron microscopy or immunofluorescence. Partitioning gradually progresses during embryogenesis, with the first dense regions appearing at gastrulation [50,51]. **(c)**. Schematic of whole-chromosome configurations during embryogenesis. Chromosome conformation with sequence specificity is best studied by chromosome conformation capture or chromosome tracing techniques (see Figure 1). Autosomes in *C. elegans* begin as barbell-like shapes in early embryogenesis with separated heterochromatic B compartment arms and a central extended A compartment. Arms then consolidate into a single B compartment at gastrulation [21,32]. Genome compartmentalization coincides with the loss of developmental plasticity and increase in cell-fate restriction in the animal.

chromatin [55], and compartment strength: A-B borders are shifted, cis and trans A-B interactions increase, while trans A-A and cis B-B interactions decrease [23]. In addition, some regions that are located near compartment borders switch their affiliation from A to B [23]. Loss of H3K9me also causes loss of lamina association [56], and this association also plays a role in chromosome compartmentalization (see below, [23,32]). However, compartments are not fully abolished in the absence of H3K9me, possibly because some HP1 remains in these animals [55]. H3K27me3 can also promote compartmentalization, and this too may compensate for loss of H3K9me [25].

Animals lacking H3K9me were previously shown to upregulate repetitive element transcription and genome instability [57], therefore it will be interesting to test in the future if spatial organization is key to repression.

One observation suggesting structure is a critical parameter is the observation that MET-2 forms nuclear condensates in the absence of its catalytic activity, and this mutated form of MET-2 is sufficient to silence a subset of its targets [53].

Territories disassemble upon loss of Polycomb and during aging

Hi-C with embryos has shown relatively few interactions between chromosomes, which suggests intra-chromosomal interactions dominate over inter-chromosomal ones, leading to discrete chromosome territories [21]. Simultaneous territory painting of all *C. elegans* chromosomes by FISH has revealed largely discrete territories in adult germ nuclei, oocytes, neurons, intestine, and epidermis [58] (Figure 1), indicating that territory organization is consistent across stages and tissues. This discreteness appears to arise through multiple mechanisms. H3K9 methylation, lamina anchoring, and dosage compensation are all required for distinct X chromosome territory volumes in intestinal nuclei [59]. In adult epidermal nuclei, the Polycomb repressive complex component *mes-3* also promotes territory discreteness; *mes-3* loss leads to increased chromosome volumes and increased intermixing between different chromosomes. Many genes are mis-regulated in the intestinal cells of *mes-3* mutants [60], raising the question of whether disrupted territory organization destabilizes normal transcriptional profiles, perhaps through improper spatial partitioning of genes and their regulatory elements. Alternatively, if the act of transcription itself can physically displace chromatin, territory disassembly may result from transcriptional deregulation.

Loss of territory distinctness in intestinal cells is also observed with ageing, indicating impaired territory regulation is linked with the functional loss of cells and tissues [58], as previously observed for heterochromatin loss [61,62]. Deformations of the nuclear lamina are observed during normal and pathological aging [63,64]. In addition, aging is associated with mild changes in H3K27me3 and more dramatic changes in H3K9me3 [65]. With age, higher levels of H3K9me3 are observed in chromosome arms and lower levels in the center A compartments, with boundaries between H3K9me3 and active modifications (H3K4me3 and H3K36me3) becoming less distinct [65]. In aged adults, H3K9me3 is added to regions that were previously modified by H3K9me2 and H3K36me3 in larval stages, suggesting that H3K9me3 is targeted to sites that were first transcriptionally active and later repressed in larvae [65,66]. This balance between H3K9me3, H3K9me2, and other modifications may contribute to longevity, with H3K9me2 associated with inherited extended lifespan [15]. The gene expression changes that accompany these alterations in histone modifications remain murky, but may include components of fatty acid metabolism and some repeat sequences [15,65,67].

Lamina functions at different scales

Radial positioning of heterochromatin and euchromatin

Lamina interactions with heterochromatin are proposed to be a major driver of radial nuclear organization, placing compact heterochromatin at the nuclear periphery and loose euchromatin in the center [41,68]. In *C. elegans*, recent studies suggest radial positioning is controlled by a handoff of mechanisms during development, and demonstrate that both heterochromatic and euchromatic factors participate in this process. Tethering heterochromatin to the lamina in *C. elegans* was previously shown to depend on multiple bridging factors such as the chromodomain protein C. elegans chromodomain protein (CEC-4), barrier-to-autointegration factor barrier to autointegration factor (BAF-1) and components of the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex [63], which parallels findings in other species [69]. Loss of *cec-4*, for example, leads to subtle delocalization of the genome and larger effects on artificial transgene arrays [56,63]. In differentiated intestinal cells, an indirect euchromatin sequestration pathway augments heterochromatin tethering by preventing the histone acetyltransferase CREB-binding protein (CBP-1)/p300 from placing aberrant H3K27ac in heterochromatic regions. Loss of the euchromatic factor mortality factor related gene (MRG-1) leads to mislocalized H3K27ac, select heterochromatic gene activation and lamina delocalization [70]. Loss of *mrg-1* also weakens the ability of cells to maintain their identity upon induced reprogramming [71], and loss of heterochromatin integrity may contribute to this phenotype.

Lamina effects on chromatin organization at different genomic scales

Historically the lamina has been linked to compact chromatin [4,41,63], implying that physical association with the lamina may participate in chromatin compaction. However, different effects on chromosome topology have recently emerged at different genomic scales, challenging this long-held assumption. At the chromosome-wide scale in embryos, HiC with mixed stages and chromosome tracing of early embryos reveal that CEC-4-mediated attachment of chrV to the lamina stretches whole chromosomes and keeps domains separated in space [23,32]. However, the HiC and chromosome tracing data are dissimilar for the smaller chrI, with loss of *cec-4* causing opposite effects on inter-arm interactions. These differing results may reflect developmental-specific effects on chrI, as it is yet unknown if the lamina plays the same role throughout embryogenesis. Altogether, these studies found that loss of anchoring causes changes in compaction of whole chromosomes, with shifting and weakening of compartment boundaries, reduction of conformational diversity, and an increase in inter-compartment mixing [23,32].

Thus, chromosome stretching in embryos differs from the classical view of lamina-induced compaction at the local heterochromatin level.

Variability in lamina-chromatin association at the single cell and gene level

The lamina has emerged as a dynamic environment, which at the global scale may appear repressive, but at the single-cell level and local scale can harbor active loci. Early population analyses in *C. elegans* found that perinuclear anchoring of chromatin (lamina-associated domains or LADs) is absent from chromosome centers and enriched along discontinuous sections of chromosome arms and repetitive transgenes [37]. However, recent single-cell analysis showed that lamina association can occur anywhere along chromosomes and is likely dynamic [32]. While the previously defined LADs showed high concordance with heterochromatin markers [37], it is also known that actively transcribed genes can also associate with the lamina-embedded nuclear pore complexes [72,73]. Moreover, recent work has found that loci bound by certain transcription factors like TFIIIC can associate with the lamina in the absence of heterochromatin markers [74]. Thus, lamina association is variable from cell to cell and gene to gene, and the lamina environment is not exclusive to silent chromatin as a rule.

In addition to variability in the wild-type context, radial positioning of heterochromatin can be modulated in response to environmental conditions. For example, heterochromatin is upregulated under starvation stress, and lamina-adjacent chromatin increases in germline cells [75]. This relocation also involves bridging proteins like CEC-4, topoisomeriase II and condensin II [75].

Outlook

Models of genome organization are being refined at a rapid rate, but the field is still in its infancy regarding *in vivo* forms and functions. The recent work we've highlighted has opened up new questions to address:

- Features like TADs and compartments likely exist as dynamic structures [35,42,43], changing from one conformation to the next, thus live imaging will be essential to measure the temporal dynamics of different conformations.
- The drivers and constraints of compartmentalization are poorly understood. Intriguing phase-separation hypotheses for chromatin compartment formation are prominent [36], but remain to be tested *in vivo*. Current top candidates for regulation include heterochromatin and lamina proteins, and these can be tested in genetically tractable model organisms.
- The relationship and the dynamics between large-scale (e.g. arm-center) and small-scale (e.g. inter-miniTAD) compartmentalization is unclear. Methodology that can measure both levels of organization robustly in single cells will be useful.
- Chromatin organization is implicated in many biological processes [76,77] but molecular mechanisms are lacking, and the field is currently dominated by correlations rather than direct causation evidence. Thus, more single-cell multimodal studies should be undertaken that simultaneously measure chromosome features *in situ* and their functional output.
- Since any particular feature's formation could be temporally separate from its phenotypes [22], a wider net should be cast when testing for physiological roles. An ambitious future goal will be to incorporate transcriptional effects with other nuclear functions at various time points for a comprehensive understanding of chromosome organization in multicellular life.

Conflict of interest statement

No conflict of interest exists.

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